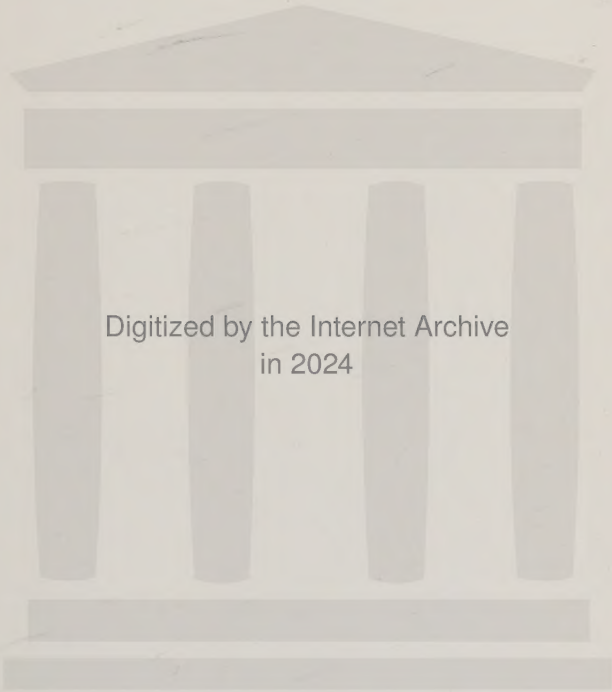
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FOOD ANALYSIS

TYPICAL METHODS

AND

THE INTERPRETATION OF RESULTS

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FOOD ANALYSIS

TYPICAL METHODS
AND
THE INTERPRETATION OF RESULTS

BY

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PREFACE

This book has grown out of the courses given to the author's students in food analysis during the last few years. Experience with these classes, which have used as textbooks mainly Leach's Food Analysis and Bulletin 107 of the Bureau of Chemistry, has shown the need of a book which should cover distinctly less ground than either of these, but should give to the student a more detailed discussion of the analytical processes involved, their suitability and limitations. Further, an attempt has been made to lay greater emphasis on the interpretation of the analytical results. To the author's mind the principal asset to be gained by the student from any detailed consideration of the methods employed to detect adulteration in foods is the exercise of judgment and the training of the sense of discrimination, which is derived from a critical balancing of the data obtained in a food analysis. Substances are being examined which are usually capable of wide natural variations in composition, and an exceptional opportunity is afforded for a critical study of the analytical factors in order to determine whether or not they imply artificial manipulation of the product.

Because the primary intention has been to write a book of the character outlined no effort has been made to include a great variety of food materials, nor necessarily those of greatest economic importance or which are most widely used. Certain typical foods have been selected to illustrate important methods of attack or characteristic methods of food analysis. In a word, the book has been written and the material selected primarily for the undergraduate student of analytical chemistry rather than for the practising chemist.

The fact that certain typical foods have been selected should not be considered as implying any intention to limit the student to the particular examples cited. Other products, similar in general character to those discussed, involving the same or different forms of adulteration, will readily suggest themselves. These

may be purchased, or sometimes more conveniently be prepared in the laboratory, and given to the student for analysis and intelligent interpretation.

Three of the most important groups of foods, fats and oils, carbohydrate foods and alcoholic beverages, have been treated at some length, general methods common to the group being taken up first in each case, followed by a more detailed discussion of several typical examples. The detection and identification of artificial colors has been treated perhaps more fully than is warranted by the actual importance of the subject because this part of the work frequently causes the student some difficulty, and adequate discussions of it are hard to find. Moreover, it affords excellent training in the detection of minute quantities of material through a systematic procedure.

The standard texts on food analysis and related subjects have been freely consulted, especially those of Leach, Browne and Sherman, as well as Allen's Commercial Organic Analysis, and much valuable material selected. Particularly helpful have been found the publications of the Bureau of Chemistry, especially the bulletins comprising the annual proceedings of the Association of Official Agricultural Chemists, these furnishing access to the best of present American work in food analysis. For these reasons the methods chosen follow most closely typical American practice, although an earnest endeavor has been made not to slight the work of European food chemists.

Acknowledgment is gratefully made to the author's many friends who have aided by encouragement and advice, and especially to Mr. G. W. Rolfe and Mr. A. L. Sullivan, who have read the chapters on Carbohydrate Foods and Alcoholic Beverages respectively and made many valuable suggestions.

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FOOD ANALYSIS

CHAPTER I

GENERAL METHODS

Preparation of the Sample.—The material will often be in proper condition for analysis as received. The main points to be considered are whether the portion to be examined is of uniform composition and whether it is finely divided. Usually the first condition may be brought about by thorough mixing and sampling of the material; for the latter it may be ground in an ordinary food chopper or pulverized in a coffee or drug mill, or if small the sample may be ground in an ordinary porcelain mortar.

Specific Gravity.—In food analysis this determination has reference almost invariably to liquids. Where only a fair degree of accuracy is desired the determination can be made conveniently and quickly by means of the Westphal balance. In principle this device is based upon the well-known law of physics that a body immersed in a liquid is buoyed up by a force equal to the weight of liquid displaced. The apparatus, shown in Fig. 1, consists of a beam balanced on a knife edge (X) and having a plummet or sinker (Y) suspended from one end and counterpoised by a fixed brass weight (Z) at the other. The distance between (X) and the point of support (W) is divided by notches into ten equal parts. The sinker, which is made of glass and provided with a thermometer, is made of such a size that it will displace exactly 5 grams of water at 15°C. or 15.5°C. The

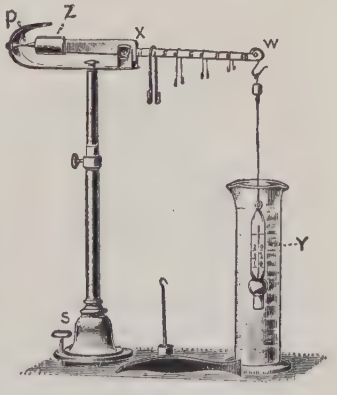


FIG. 1.—Westphal balance.

plummet and the hook from which it is suspended are made of a definite weight, usually 15 grams, so that they are interchangeable in different instruments.

In using the balance it is first adjusted by means of the leveling screw (*S*) until the pointer (*P*) on the arm is exactly opposite the reference point. If now the sinker be entirely immersed in distilled water at the standard temperature, it will require a weight of 5 grams at (*W*) to make it balance again. For liquids heavier than water a greater weight will be needed and for liquids lighter than water one correspondingly less. For reading the decimals of a unit of specific gravity, use is made of the notched divisions on the beam. Thus if the 5 gram weight placed directly over the plummet shows a specific gravity of 1.0000, the same weight placed 0.3 of the distance on the beam would correspond to a gravity of

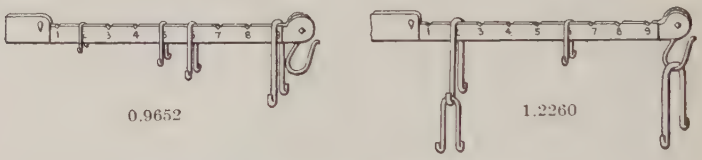


FIG. 2.—Reading the Westphal balance.

0.3000. The second decimal is obtained by means of a 0.5 gram weight, the third by a 0.05 gram weight and the fourth by a 0.005 gram weight. Typical examples of the method of reading the instrument are shown in Fig. 2.

For more exact determinations of specific gravity some form of pyknometer should be used. Reduced to its lowest terms this apparatus consists of a light container for weighing equal volumes of liquids measured accurately at a definite temperature. Two common forms are the Sprengel-Ostwald tube (Fig. 3) and the specific gravity bottle (Fig. 4).

The Sprengel Tube.—This is especially useful when only a small amount of liquid is available for the determination, or when the determination is to be made at a temperature quite different from room temperature. The best form is the one suggested by Ostwald and shown in the figure, one arm being a capillary tube while the other holds the bulk of the liquid. In using it the end (*B*) should be dipped into the liquid, which has been cooled to several degrees below the desired temperature, and the

tube filled by applying suction through a rubber tube attached to the tip (*A*). The pyknometer is then suspended in a narrow beaker filled with water at the desired temperature and when its contents have attained this temperature, as shown by the meniscus in the capillary remaining stationary, by touching the tip (*A*) with the edge of a filter paper the meniscus can be brought to the reference mark (*C*). The pyknometer is then carefully wiped dry, suspended from the hook over the balance pan and weighed. It is necessary also to calibrate the pyknometer, which is done by weighing it empty and dry, then weighing it filled with recently boiled distilled water in the manner just described.

The Specific Gravity Bottle.—These can be procured of various sizes, most conveniently of 25 or 50 cc. capacity, provided with a thermometer stopper as shown, extending to 40°C. One should be chosen with as large a cap to the side tube as possible and it is best to have a very small hole drilled or blown in the ex-

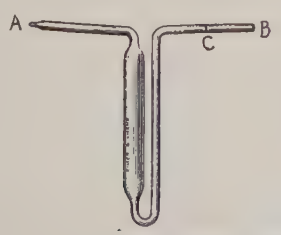


FIG. 3.—Sprengel-Ostwald tube.

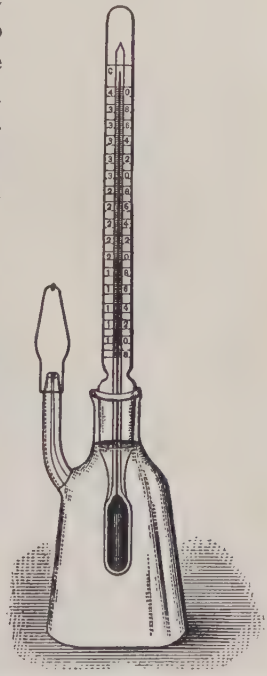


FIG. 4.—Specific gravity bottle—Geissler form.

tremity of the cap so that the liquid may expand into the cap during the weighing without forcing it up and causing leakage. The thermometers are not always accurate and for careful work should be tested by comparison with one of known accuracy.

Calibration of the Pyknometer.—This is done by weighing the pyknometer empty and dry and then filling it with recently boiled distilled water at a temperature one or two degrees below that at which the determination is to be made. The pyknometer should

be filled nearly full with the water so that when the thermometer is inserted the water shall overflow through the capillary. The bottle is then placed in a bath of water kept at the desired temperature, and as soon as its contents have reached that temperature, as shown by the thermometer, the excess of water is carefully wiped from the tip of the capillary, the cap placed on and the bottle wiped dry and weighed. Subtracting the weight of the pyknometer gives the water content of the bottle at the temperature used, which is most conveniently 20°C., although 15.6°C. is often employed. The determination should be repeated and the mean of two closely agreeing values recorded for use.

To determine the specific gravity of a liquid, the pyknometer thus calibrated is rinsed several times with the liquid with which it is to be filled, or it may be rinsed with alcohol and then with ether and dried in a water oven. (If this is done care should be taken that the thermometer is not put into the oven also, since this, registering to only 40°, will be broken.) The pyknometer is then filled with the liquid, using the same precautions as regards temperature as with distilled water, and weighed. The weight of the liquid contained, divided by the water content, is the specific gravity.

In stating the specific gravity of a liquid it is advisable to record the temperature at which the determination was made as well as the temperature of the water with which it is compared. This is done in the form of a fraction thus, $\frac{20°}{20°}$, meaning the specific gravity at 20°C. referred to water at 20°C.

In order to determine the specific gravity at a given temperature $t°$, referred to water at its maximum density 4°C., the value determined at $\frac{t°}{t°}$ should be multiplied by the density of water at $t°$, taken from Table I, page 6.

Thus the value for $\frac{20°}{20°}$ must be multiplied by 0.998234 to obtain the value at $\frac{20°}{4°}$.

Index of Refraction.—When a beam of light passes obliquely from one medium to another of different optical density it is bent out of its course or *refracted*. For two given media the amount of

this refraction is a constant for any definite temperature and can be stated mathematically by the expression $\frac{\sin i}{\sin r} = n$, where i is the *angle of incidence* made by the incident ray with the perpendicular to the dividing surface and r is the *angle of refraction* made by the refracted ray with the perpendicular; n is called the index of refraction. For example, in Fig. 5 let AB be the surface of separation between two media, of which the upper is the rarer, and let a beam of light pass through in the direction IO. The angle ION, which the incident ray makes with the perpendicular, is the angle of incidence, and the angle RON', made by the refracted ray with the perpendicular, is the angle of refraction. The index of refraction would be represented in the figure by the ratio $\frac{ab}{cd}$.

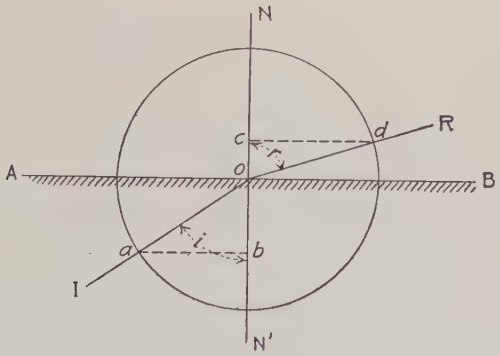


FIG. 5.—Illustrating the law of refraction.

Ordinarily the index of refraction of a substance is taken as the ratio of the angles formed when light passes from *air* to the substance and is referred to the D ray of the spectrum as the standard wave length of light, so that for a temperature of 20°C. n would be written n_D^{20}.

The Critical Angle and Total Reflection.—Referring again to Fig. 5, it will be seen that if the light pass in the opposite direction, from the denser to the rarer medium, the angle of refraction ION will be greater than the angle of incidence RON'. If the angle of incidence be increased, then at a certain angle of incidence the angle of refraction will become 90°, that is, the refracted

TABLE I.—DENSITY OF PURE WATER FREE FROM AIR

[Under standard pressure (76 cm.), at every tenth part of a degree of the international hydrogen scale from 0° to 40°C., in grams per milliliter[1]]

Degrees Centigrade	Tenths of degrees									
	0	1	2	3	4	5	6	7	8	9
0	0.999 868	874	881	887	893	899	905	910	916	921
1	926	931	936	940	945	949	953	957	961	964
2	967	971	974	976	979	982	984	986	988	990
3	992	993	995	996	997	998	998	999	999	*000
4	1.000 000	*999	*999	*999	*998	*997	*997	*996	*994	*993
5	0.999 991	990	988	986	984	981	979	976	974	971
6	968	965	961	958	954	950	946	942	938	934
7	929	924	920	915	910	904	899	893	888	882
8	876	870	864	857	851	844	837	830	823	816
9	809	801	794	786	778	770	762	753	745	736
10	728	719	710	701	692	682	672	663	653	643
11	633	622	612	602	591	580	569	558	547	536
12	524	513	501	489	478	466	453	441	429	416
13	404	391	378	365	352	339	325	312	298	285
14	271	257	243	228	214	200	185	171	156	141
15	126	111	096	080	065	049	034	018	002	986
16	0.998 970	954	937	921	904	888	871	854	837	820
17	802	785	768	750	732	715	697	679	661	642
18	624	605	587	568	549	530	511	492	473	454
19	434	415	395	375	355	335	315	295	275	254
20	234	213	193	172	151	130	109	087	066	044
21	023	001	*979	*958	*935	*913	*891	*869	*847	*824
22	0.997 801	779	756	733	710	687	664	640	617	593
23	570	546	522	498	474	450	426	402	377	353
24	328	303	279	254	229	204	178	153	128	102
25	077	051	025	*999	*973	*947	*921	*895	*868	*842
26	0.996 815	789	762	735	708	681	654	627	600	572
27	545	517	489	462	434	406	378	350	321	293
28	265	236	208	179	150	121	092	063	034	005
29	0.995 976	946	917	887	857	828	798	768	738	708
30	678	647	617	586	556	525	495	464	433	402
31	371	340	308	277	246	214	183	151	119	088
32	056	024	*992	*959	*927	*895	*863	*830	*797	*765
33	0.994 732	699	666	633	600	567	534	501	467	434
34	400	367	333	299	265	231	197	163	129	095
35	061	026	*992	*957	*923	*888	*853	*818	*783	*748
36	0.993 713	678	643	607	572	536	501	465	430	394
37	358	322	286	250	214	178	141	105	069	032
38	0.992 996	959	922	885	849	812	775	738	700	663
39	626	589	551	514	476	438	401	363	325	287
40	249	211	173	135	097	058	020	*981	*943	*904

[1] According to P. Chappuis, Bureau international des Poids et Mesures, Travaux et Mémoires, XIII, 1907.

* The asterisk indicates a diminution of one in the third place decimal.

ray will coincide with the dividing surface. For incident rays striking the surface at a greater angle than this, the beam of light will be *totally reflected* and there will be no refracted ray. The angle of incidence at which this occurs is known as the *critical angle.*

Then since $n = \frac{\sin i}{\sin r}$,

at the critical angle

$$n = \frac{\sin i}{\sin 90^\circ} = \frac{\sin i}{1} = \sin i$$

That is, in passing from a denser to a rarer medium, the index of refraction is equal to the sine of the angle of incidence for the border line of total reflection.

The forms of refractometer most commonly employed in food analysis are based upon this principle of measuring the angle of incidence for total reflection.

Abbe Refractometer.—In this instrument the refractive index of a liquid is determined by measuring the critical angle for light passing into it from a glass prism of higher refractive index. The sine of this angle is the index of refraction of the liquid referred to glass and this, multiplied by the refractive index of the glass, gives the index of refraction of the liquid, referred to air.

The apparatus, Fig. 6, consists essentially of three parts:

(*a*) Two prisms (*A*) and (*B*) of flint glass, having a refractive index of 1.75, mounted so that they can be separated and a few drops of the liquid to be examined placed between, forming a thin layer when the prisms are joined again. The prisms can be rotated by means of a movable arm or alidade (*C*) which carries the reading magnifier (*M*).

(*b*) A telescope (*T*) provided with cross-hairs by which the position of the border line of total reflection can be observed.

(*c*) The sector (*S*) divided proportionally to the sines of the various angles of incidence for the border line of total reflection, and therefore representing indices of refraction.

An important part of the apparatus is the compensator, placed in the tube of the telescope at (*P*) and composed of two similar Amici prisms which can be rotated simultaneously in opposite directions by the milled head (*H*). In this way the compensator can be given an equal but opposite dispersion to that of the liquid

under examination, and the border line of total reflection, which may appear as a colored band, can be changed to a sharp colorless line.

To use the refractometer, the prisms and telescope are rotated as far as possible in the direction from the observer, the latch is turned to the right and the prism (*B*) swung open. Two or three drops of the liquid to be examined are then placed on the polished surface of the stationary prism (*A*) and prism (*B*) brought back into position and clamped as before. The whole instrument is then rotated toward the observer and the mirror (*G*) turned so as to reflect the light upon the prism (*B*).

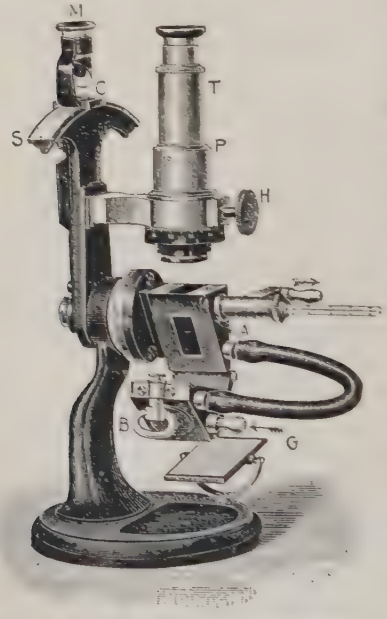


FIG. 6.—The Abbe refractometer.

By means of the movable arm (*C*) the prisms are rotated in the field of the telescope until the border line of total reflection is observed, the lower portion of the field being dark and the upper portion bright. If the border line should be colored, this is corrected by means of the compensator, the eyepiece of the telescope focused sharply, and by cautious movement of the arm the line made to coincide with the junction of the cross-hairs. The reading of the scale through the magnifier (*M*) gives directly the refractive index to the fourth decimal. The temperature should be read by the thermometer to the nearest $\frac{1}{2}°$ before reading the index. After several settings and readings of the instrument have been made the prisms should be cleaned with alcohol and a soft cloth.

Temperature Regulation.—The refractive index of liquids is a function of the temperature, increasing as the temperature decreases. It is hence necessary to note the temperature at which

the reading is made. Moreover, with some substances, as the solid fats, it is essential that the temperature of observation should be above their melting points, so that some method of controlling and raising the temperature is desirable. This is done in the Abbe instrument by a current of water of the desired temperature which flows through the prism casing in the direction of the arrows shown in Fig. 6. This water may be supplied from a large reservoir of warm water, or the spiral heater supplied by the mak-

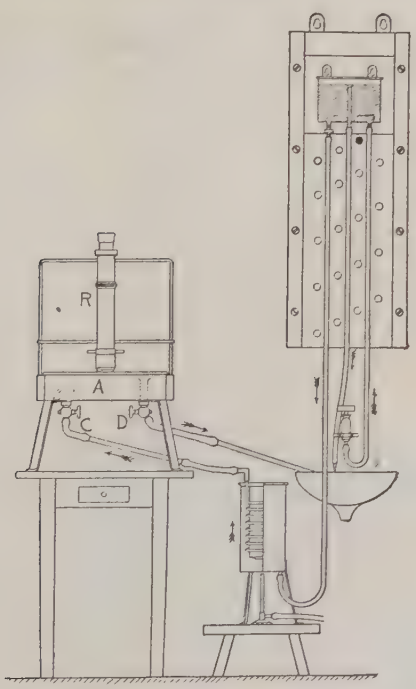


FIG. 7.—Refractometer heater.

ers may be employed, as shown in Fig. 7 connected with the immersion refractometer. The temperature at which the determination was made should always be stated in reporting a reading.

The correctness of the adjustment of the instrument should be tested from time to time. This may be done by taking the average of several readings on distilled water, the refractive index of which for ordinary temperatures is shown in Table II.

TABLE II.—REFRACTIVE INDEX OF WATER

Temperature, °C.	Refractive index	Temperature, °C.	Refractive index
18	1.3332	23	1.3327
19	1.3331	24	1.3326
20	1.3330	25	1.3325
21	1.3329	26	1.3324
22	1.3328	27	1.3323

The makers of the instrument furnish with it a glass test plate of known refractive index which may also be used. For the method of employing it the circular which comes with the refractometer

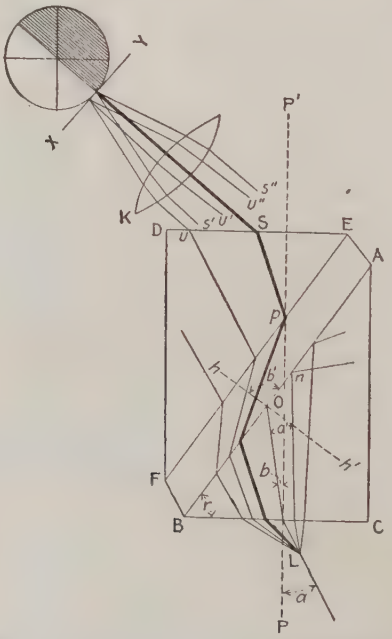


FIG. 8.—Diagram of Abbe refractometer.

may be consulted, or reference may be made to Browne: Handbook of Sugar Analysis, page 59. The Abbe instrument, if in adjustment, should give the index of refraction to 0.0002.

Figure 8, from Browne's Handbook of Sugar Analysis, illustrates diagrammatically the passage of light through the instru-

ment. The heavy line represents the border line of total reflection, the light striking the surface $A\ B$ at a less angle being refracted and illuminating the field of the telescope. The rays which fall upon the surface at a greater angle are totally reflected, leaving the corresponding portion of the telescopic field dark.

The Immersion Refractometer.—This instrument, of later construction than the Abbe refractometer, is capable of considerably

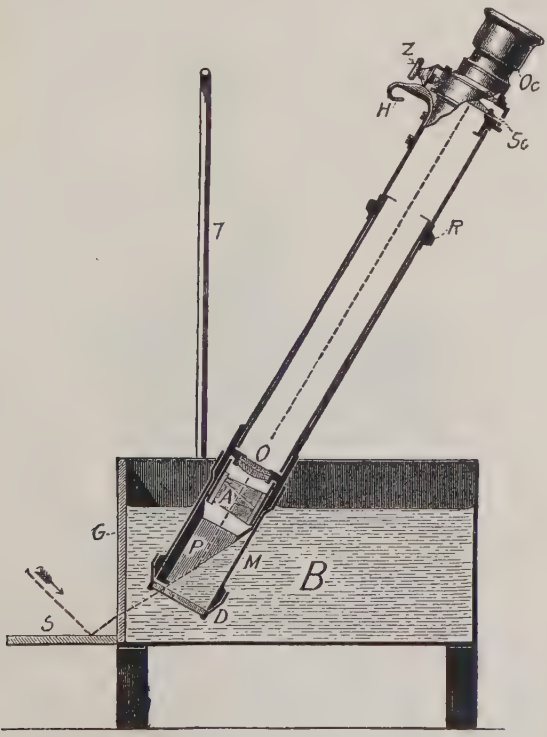


FIG. 9.—Construction of immersion refractometer.

greater delicacy. It is, on the other hand, more limited in its range, giving indices of refraction between 1.32 and 1.36 only.

The principle on which it is based is the same as in the Abbe instrument, depending upon the observation of the border line of total reflection.

Referring to Fig. 9, light is reflected from a mirror (S) so as to

pass nearly parallel to the oblique surface of the prism (*P*). Rays which coincide with this surface form the border line of total reflection and are refracted upward through the prism in some such direction as the dotted line *PO*. Rays of light which strike the surface obliquely are refracted and illuminate the upper portion of the field. Since there can be in the prism no greater angle of refraction than that for the border line of total reflection, the lower portion of the field remains dark.

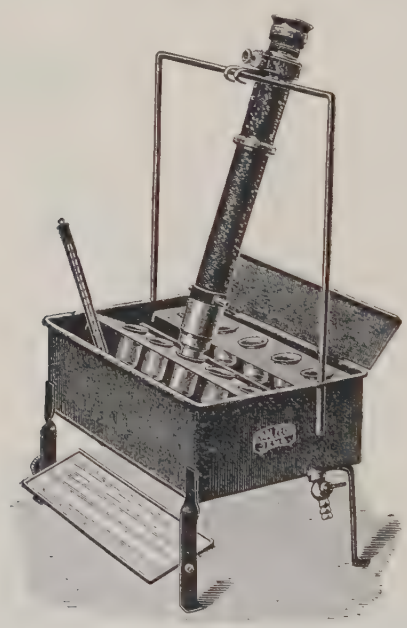


FIG. 10.—Method of using immersion refractometer.

The construction of the refractometer is clear from the figure. (*P*) is the glass prism and at the other end of the tube is the ocular (*Oc*). At (*Sc*) is the scale on which is read the degree of refraction. At (*A*) is a compensator of the same nature as that in the Abbe instrument and serving the same purpose. This is connected by an inside sleeve with the milled ring (*R*), by which it can be regulated. At (*Z*) is an ingenious vernier by which fractional parts of a degree may be read on a revolving drum.

In using the refractometer the solution to be examined is con-

tained in a small beaker kept in a bath at constant temperature, as shown in Fig. 10, or the solution is more conveniently placed in the small metal cup which may be attached directly to the end of the instrument as (*M*) in Fig. 9. The temperature of the bath should be noted by an accurate thermometer within 0.1° The refractometer is hung from the wire frame so that the light from a window or a lamp is reflected by the mirror upward through the ground glass plate in the bottom of the bath and through the prism. The milled ring (*R*) is turned until the border line is free from color and the ocular (*Oc*) focused so that the line and the scale are both sharp. The upper portion of the field will be bright and the lower portion dark, the position of the border

TABLE III.—REFRACTIVE INDICES (n_D) CORRESPONDING TO READINGS OF THE IMMERSION REFRACTOMETER

Scale reading		Scale reading		Scale reading		Scale reading		Scale reading	
0	1.32736	20	1.33513	40	1.34275	60	1.35021	80	1.35750
1	1.32775	21	1.33551	41	1.34313	61	1.35058	81	1.35786
2	1.32814	22	1.33590	42	1.34350	62	1.35095	82	1.35822
3	1.32854	23	1.33628	43	1.34388	63	1.35132	83	1.35858
4	1.32893	24	1.33667	44	1.34426	64	1.35169	84	1.35894
5	1.32932	25	1.33705	45	1.34463	65	1.35205	85	1.35930
6	1.32971	26	1.33743	46	1.34500	66	1.35242	86	1.35966
7	1.33010	27	1.33781	47	1.34537	67	1.35279	87	1.36002
8	1.33049	28	1.33820	48	1.34575	68	1.35316	88	1.36038
9	1.33087	29	1.33858	49	1.34612	69	1.35352	89	1.36074
10	1.33126	30	1.33896	50	1.34650	70	1.35388	90	1.36109
11	1.33165	31	1.33934	51	1.34687	71	1.35425	91	1.36145
12	1.33204	32	1.33972	52	1.34724	72	1.35461	92	1.36181
13	1.33242	33	1.34010	53	1.34761	73	1.35497	93	1.36217
14	1.33281	34	1.34048	54	1.34798	74	1.35533	94	1.36252
15	1.33320	35	1.34086	55	1.34836	75	1.35569	95	1.36287
16	1.33358	36	1.34124	56	1.34873	76	1.35606	96	1.36323
17	1.33397	37	1.34162	57	1.34910	77	1.35642	97	1.36359
18	1.33435	38	1.34199	58	1.34947	78	1.35678	98	1.36394
19	1.33474	39	1.34237	59	1.34984	79	1.35714	99	1.36429
								100	1.36464

line upon the graduated scale showing the index of refraction. If the line comes between two of the scale divisions its exact position may be read by the micrometer (*Z*) as follows: First, with the micrometer set at 0 read the next lower whole division on the scale, then turn the micrometer screw until the line coincides exactly with a scale division. From the graduated drum of the micrometer the exact reading may be made to hundredths of a division. The reading should be the average of several settings and should not be taken until the instrument has been kept for at least 10 minutes at the temperature of the bath. The reading may be reported directly in scale divisions or may be converted to the corresponding refractive index by Table III, page 13.

Adjusting the Refractometer—This may be done conveniently by taking a reading on distilled water, which should read 14.5 on the scale at 20°C. The same precaution should be observed as described above in regard to attaining the temperature and (*R*) should be set at 5. It will perhaps be better to have the reading on water precede the actual determination both for the practice in using the instrument and to ascertain if it is correctly adjusted. If a slight error in reading is found the instrument may be readjusted by a change in the micrometer screw. Directions for doing this will be found in Browne (*loc. cit.*) or in Leach: Food Inspection and Analysis (second edition), but it will be advisable for the student to make the necessary corrections rather than attempt this. In every case, after being used the prism should be carefully wiped dry with a soft clean cloth, and since the instrument is necessarily moved about the unprotected prism must be handled *with the greatest possible care.*

Determination of Moisture.—The method most commonly employed for the determination of moisture in foods is by drying at the temperature of boiling water.

A convenient weight, usually 2–10 grams, is spread in a thin layer in a flat-bottomed dish or on a watch-glass and dried in an oven surrounded by boiling water until the weight, at intervals of half an hour, remains constant. The loss in weight is taken as water.

A convenient copper oven which serves both for drying and for evaporating is shown in Fig. 11, dishes of various sizes being

placed on copper rings over the openings and the drying being done on shelves within the oven. A constant level tube may be provided at one end, to be connected with the water supply. This

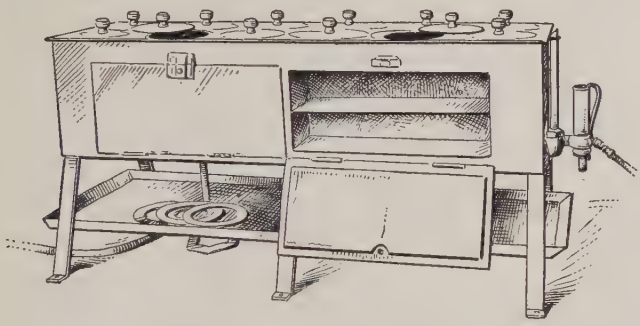


FIG. 11.—Oven and water-bath.

particular bath is heated by lamps beneath, although a steam coil within the bath itself can be used to advantage. Usually a period of from 2 to 5 hours is sufficient to dry the sample.

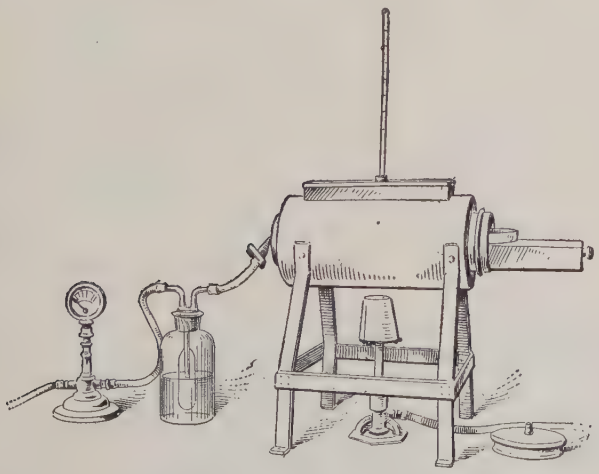


FIG. 12.—Vacuum drying oven.

There are several precautions to be observed and the exact details of time, temperature, etc., depend somewhat upon the character of the material to be dried.

With some substances water is expelled rather slowly at the temperature of boiling water, which rarely reaches 100°C., and in such cases an air oven heated to 105°C. is preferable. On the other hand, in some cases the temperature of boiling water is too high for correct results. Levulose is decomposed in the presence of water at temperatures in excess of 70°C., so that the method cannot be used for such products as honey, jams and fruit juices, which contain this sugar in appreciable amounts. Such materials should be dried at 70°C. *in vacuo* or since many laboratories are not equipped with the necessary apparatus for this, densimetric or refractometric methods are commonly employed. These, being generally used for carbohydrate foods, are discussed in Chapter VI. A simple form of copper oven as used in the author's laboratory for drying in a partial vacuum or in a current of inert gas is shown in Fig. 12.

A still simpler form of vacuum oven, suitable for small amounts of substance and which can be made from laboratory materials, is described by Browne.[1]

Further, if the food material contain other substances than moisture that are volatile at 100°C., they would be included in the moisture determination made in the manner indicated. Such a case would be the volatile oil in spices.

If it is desired to dry liquid foods that are thick or sirupy they should be spread upon some absorbent material in the dish, as sand, asbestos or pumice.

Determination of Ash.—It is helpful with many foods to determine not only the total ash but the ash soluble and insoluble in water, the alkalinity of the soluble ash, and the proportion of ash insoluble in acids.

Total Ash.—This determination may be made on the sample used for the estimation of moisture, or a fresh portion may be taken. In either case, the sample should be in a weighed platinum dish, preferably flat-bottomed, and should be ignited gently over a small flame until thoroughly charred. The dish is then placed in a muffle and heated to low redness until a white ash is obtained, when it is cooled in a desiccator and weighed.

A convenient muffle, capable of taking several small dishes, is shown in Fig. 13. When it is desired to regulate the temperature

[1] Handbook of Sugar Analysis, p. 23.

more closely an electric muffle furnace, as shown in Fig. 14, will be found advantageous.

The essential point to bear in mind in this determination is that the ash should not be heated to too high a temperature. There is danger that volatile material, as chlorides of the alkalies, will be driven off, or on the other hand, that a portion of the ash will be fused and enclose carbon which will thus escape ignition. If the ignition is made in a muffle the temperature should be so regulated that it does not exceed 600° to 650°C.; if by direct heating of the dish the latter should be nowhere heated to more than

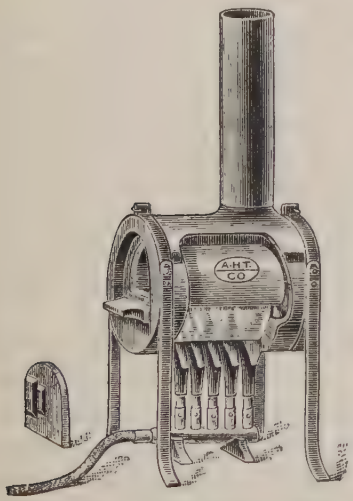


FIG. 13.—Gas-heated muffle furnace.

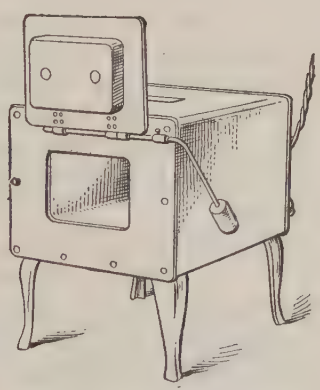


FIG. 14.—Electrically-heated muffle furnace.

just visible redness. The ash determination, like the moisture, although apparently very simple, is in reality difficult to make satisfactorily. A good ash determination may require several hours and the resultant ash should be of a uniform white or gray color, occasionally reddish or green, and free from fused lumps or particles of unburned carbon.

If it is difficult to secure a white ash in this manner, the mass should be treated several times with hot water and filtered through an ashless filter. The filter and residue are ignited in the original dish, the filtrate added to the dish, evaporated to dryness on the

water-bath, and the whole ignited for a few moments at a low red heat.

Sirups or foods which contain much water must be heated carefully. Not over 5–10 grams should be taken and a dish of at least 50 cc. capacity is best. The dish should be heated cautiously with a low flame until the material begins to char, then by placing the flame at one side of the dish it is generally possible to regulate the charring so that it shall spread gradually over the dish without excessive foaming. A bit of vaseline placed on the mass is sometimes a considerable help and adds nothing to the ash.

The use of a muffle although convenient is not essential, for the ashing will usually proceed properly if the dish is heated directly with a small flame, in a place free from draughts and if the material be stirred from time to time with a stout platinum wire. Covering the dish loosely with a sheet of platinum foil hastens the process by reflecting heat down upon the material.

Caution is also necessary in handling the dish containing the ash on account of the light, fluffy, skeleton ash often obtained, which is easily blown from the dish. While carrying the latter, and even while it is in the desiccator, it is best covered with a small watch-glass.

Soluble and Insoluble Ash.—To determine the *ash insoluble in water*, after weighing the total ash, add about 25 cc. of water to the dish, cover it with a watch-glass to avoid loss by spattering, and heat it nearly to boiling. Filter through an ashless filter and wash with an equal volume of hot water. Place the filter paper and residue again in the dish, ignite and weigh. From the weight calculate the water-insoluble ash and by difference the *water-soluble ash.*

Alkalinity of Water-soluble Ash.—Some food products contain notable amounts of organic acids, which during ignition become converted in part into carbon dioxide, held by the alkaline salts of the ash as carbonates, largely as potassium carbonate. A decreased quantity of alkali salts in the ash would hence be of some importance as indicating adulteration. On the other hand, an increase in the alkaline character of the ash may indicate chemical treatment, as in the case of cocoa. The determination of the alkalinity of the ash may therefore be of decided value. The determination is carried out in the following manner:

Allow the filtrate from the insoluble ash to cool and titrate with $\frac{N}{10}$ hydrochloric acid, using methyl orange as an indicator. Report the result as the number of cubic centimeters of $\frac{N}{10}$ acid required to neutralize the ash of 1 gram of sample.

Alkalinity of Insoluble Ash.—To determine this add 15 cc. of $\frac{N}{10}$ hydrochloric acid to the weighed insoluble ash in the platinum dish, cover with a watch-glass and heat cautiously nearly to boiling, over a small flame. Allow it to cool and titrate the excess of acid with $\frac{N}{10}$ sodium hydroxide, using methyl orange as before. The result is expressed as in the previous determination.

Ash Insoluble in Acid.—This determination is of value in showing added mineral matter, such as dirt or sand in spices, talc in confectionery, etc. The weighed residue in the determination of ash insoluble in water may be used, or if this has been used in the determination of alkalinity, a fresh 2 gram portion of the original sample may be ignited and used directly. To the ash in the dish, in either case, add 25 cc. of 10 per cent. hydrochloric acid (sp. gr. 1.050), cover with a watch-glass and boil gently over a low flame for 5 minutes, filter through an ashless filter, wash with hot water, return the filter and residue to the dish, ignite and weigh.

Colorimetric Determinations.—For the determination of some constituents present in small amount, for instance citral in lemon extracts or vanillin in vanillas, methods depending upon the quantitative determination of color are frequently used.

Measurements of a fair degree of accuracy can be made by comparing the colors directly in tubes with smooth polished bottoms, as the ordinary Nessler tubes used in water analysis; or two ordinary graduated cylinders can be selected which are of about the same diameter and have clear glass in the bottoms, free from distortion. If one of these is used for the sample to be examined and the other contains a standard solution of nearly the same degree of color, then if small portions of the deeper colored solution are removed until the colors are of equal intensity, the strength of the two solutions will be inversely proportional to the heights at which they stand in the cylinders. Somewhat more

convenient is a pair of Hehner's colorimeters (Fig. 15) which are used in exactly the same way.

It is important, and the same holds true of more elaborate colorimeters, that the standard and the unknown solution should be reasonably close to the same intensity of color, since for some substances the depth of color is not exactly proportional to the strength of the solution for all concentrations. It is always advisable to test the accuracy of the comparisons under the conditions of the experiment by dividing a colored solution into two exactly equal parts, placing them into the two cylinders and noting the readings. It is also best to check the reading with the relative position of the cylinders reversed, since some observers invariably see the right- or left-hand tube deeper in color than the other.

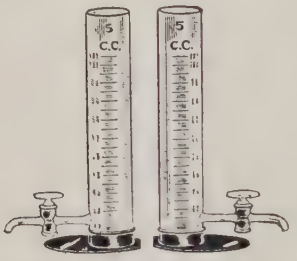


FIG. 15.—Hehner's colorimeters.

For exact work some more elaborate colorimeter should be used. A typical one, giving very accurate results, is the Duboscq colorimeter, shown with its case removed in Fig. 16. The liquids to be compared are placed in the two cylinders with plane and polished bottoms. The cylinders can be raised or lowered so that the depth of liquid examined is in each case that between the bottom of the interior glass column and the bottom of the cylinder. This depth can be read to 0.1 mm. by a scale and vernier at the back. Light is reflected through the solutions by the mirror, on the reverse of which is a milk-white reflector, which can be substituted for the mirror if desired. The light is reflected by two prisms in the upper portion of the instrument, into the telescope, the field of which appears divided into two equal parts, permitting an exact comparison of the two colors.

The path of the rays of light in the instrument will be readily understood from the diagram in Fig. 17.

If a = the reading of the scale for the standard solution,
b = the reading of the scale for the unknown solution,
P = the per cent. of substance in the standard solution, and
X = the per cent. of substance in the unknown solution,

then

$$X = \frac{aP}{b}$$

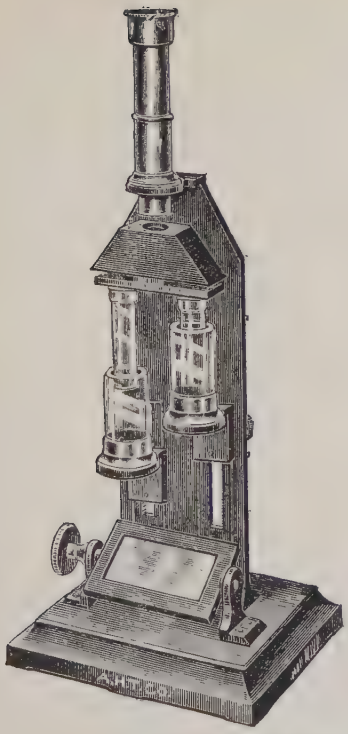


Fig. 16.—Duboscq colorimeter.

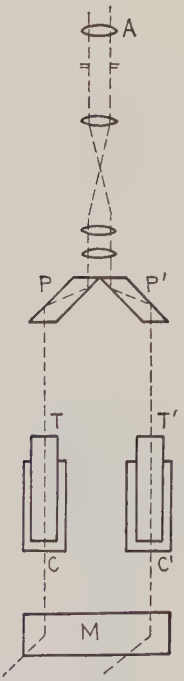


Fig. 17.—Diagram of Duboscq colorimeter.

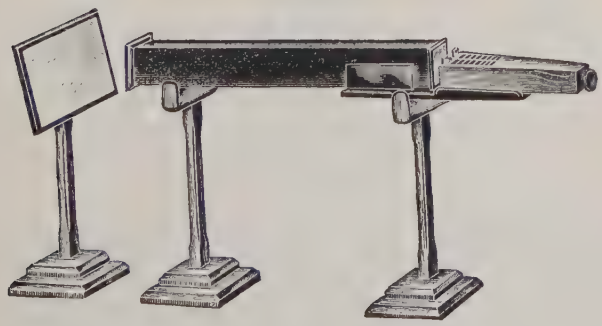


Fig. 18.—Lovibond tintometer.

If it is desired to determine not so much the amount of color as its *character*, a tintometer should be used rather than a colorimeter. Such a determination is given under Vanilla Extract, page 389, and the use of the Lovibond Tintometer is suggested. This instrument, shown in Fig. 18, consists essentially of a narrow box provided with an opening for the eye through which comparison may be made of the liquid and a series of colored glasses. By inserting the proper glasses, which come ordinarily in standard sets of red, blue and yellow, the exact character of the color may be determined and recorded.



FIG. 19.—Soxhlet fat extraction apparatus

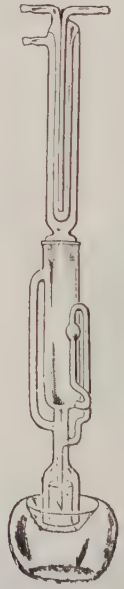


FIG. 20.—Extraction apparatus with mercury joint.

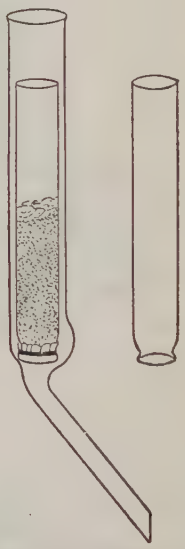


FIG. 21.—Johnson extraction apparatus.

Extraction Methods.—For such determinations as fat in cereals or volatile oil in spices, where the substance is slowly removed by a proper solvent, some form of continuous extraction apparatus is essential. The one most commonly employed is the Soxhlet extractor, as shown in Fig. 19, its method of use being obvious. The connection between the tared flask (*F*) and the extractor, as

well as the upper connection, may be made by a soft, well-chosen cork. There is, however, more or less danger that resinous matter may be extracted from the corks by the solvent employed, so that it is preferable to cover them closely with tin foil. The form shown in Fig. 20, provided with an upper ground glass joint and connected to the flask by a mercury seal instead of a cork, is better in some ways but more fragile and more expensive to replace if broken. The Soxhlet apparatus has the disadvantage that it is very wasteful of the solvent, at least 50 or 60 cc. being ordinarily required for the extraction to proceed at all.

For the extraction of volatile oil in spices the writer has used with much satisfaction the Johnson extractor, described by Winton in the second edition of Leach's Food Analysis and shown in Fig. 21. This has the advantage that only a small amount of ether, usually 10 cc., is needed, so that it can be used by large classes or for numerous determinations without the labor of preparing large quantities of anhydrous ether. The fact that very small amounts of solvent are used is of help also when this is evaporated, since the evaporation takes less time and there is less danger of loss of volatile oil, which is not the case when large quantities of ether must be evaporated.

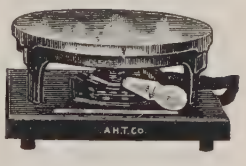


FIG. 22.—Electric hot plate.

As a source of heat the free flame must be avoided on account of the inflammable nature of the solvents usually employed. A steam bath may be used or the small electric heaters shown in Fig. 22, are especially convenient. It should be borne in mind, however, that solvents such as ether or petroleum ether should not be evaporated in any quantity in an open dish even on the electric heater, since some forms of these will ignite the vapors.

Method.—The dried material is placed in an "extraction thimble" or wrapped in several thicknesses of paper and placed in the tube of the extractor, which is then connected with the condenser. A plug of cotton should be placed in the top of the thimble to prevent the pulverized sample being carried out during the siphoning of the solvent. Sirupy or viscous materials may be dried on some absorbent substance in a tin-foil dish, which is then crumpled up and placed in the extractor. The

needful quantity of solvent is then placed in the weighed flask (*F*) which is joined to the apparatus. The time of heating may be from 2 to 20 hours, varying with the material and the solvent. The heating should be such that the solvent siphons over about five or six times an hour.

After the extraction is complete the thimble is removed, the apparatus again connected and the flask heated again until the tube of the extractor is nearly filled with the solvent, which may thus be recovered. The remainder is evaporated *almost* to dryness, conveniently by suction while on the electric heater. The last traces of solvent are removed on the water-bath and the flask and contents dried in the water-oven to constant weight.

Choice of Solvent.—Extraction of food materials is ordinarily made with either anhydrous ethyl ether or petroleum ether—that is, a low-boiling distillate from petroleum (best between 35°–45°C.). Of these two the petroleum ether is the cheaper and requires no special preparation beyond a possible fractionation to secure material boiling within the desired limits. It has the advantage also that it is not affected by traces of moisture that may be present in the material to be extracted and does not take up moisture during the extraction.

Ethyl ether, on the other hand, has the advantage that it is a better solvent for the fat than is petroleum ether, and practically all the existing standards for food analysis are based on the use of this solvent. It has the disadvantage that it must be specially freed from water and kept so during the determination, since moist ether will dissolve sugar and other material which should not be included in the true ether extract.

Preparation of Anhydrous Ethyl Ether.—Wash ordinary ether with several portions of distilled water to remove alcohol, and allow it to stand with solid potassium hydroxide or calcium chloride until apparently all the water has been removed. Decant or filter into a dry bottle and remove the last traces of moisture by adding pieces of *clean,* freshly cut sodium. Allow the ether to stand over the sodium until there is no further evolution of hydrogen, keeping it protected from the moisture of the air by a stopper carrying a calcium chloride tube. The ether may be drawn off with a pipette as wanted for use.

A rapid method for determining ether extract in such substances

as cocoa, spices, etc., has been described by Leach and Hiltner[1] This method on dry, powdered material yields results comparable with the usual continuous extraction methods and in a fraction of the time. It is carried out as follows:

Weigh 2 grams and transfer to a small beaker of at least 100 cc. capacity. Treat with about 20 cc. of ordinary ether and rotate several times, taking care to avoid loss. Let stand a few minutes and filter through a quick-acting filter into a small tared flask. Wash out the beaker with a stream of ether from a wash bottle and wash the filter with ether until the filtrate measures about 75 cc., taking care to wash the top of the filter. Place the open flask with the ether extract in a warm place where the temperature is 40° to 50°C., and allow it to remain until the ether is evaporated, usually about 17 to 18 hours. Cool and weigh.

Note.—The essential things to avoid in using this method successfully are the crawling of the extract and the evaporation of the ether. Best results will be obtained by the use of a rather wide-mouthed flask for evaporating the ether, and in the writer's laboratory better results have been obtained by filtering the ether on a thin felt of asbestos in a Gooch crucible rather than on a filter. This may be prepared in the usual manner, washed with alcohol and with ether, and sucked dry before using. Only enough suction should be applied to give fairly rapid filtration without hastening evaporation. Suction cannot well be applied in evaporating the ether without causing the condensation of atmospheric moisture, especially on days of high humidity.

Determination of Nitrogen.—Nitrogen determinations in foods are practically always made by some form of the Kjeldahl or moist combustion process. This method, which was originally devised by Kjeldahl[2] for the determination of nitrogen in beers, is based upon the decomposition of the nitrogenous material by boiling it with strong sulphuric acid. The carbon and hydrogen of the material are oxidized to carbon dioxide and water, a portion of the sulphuric acid being reduced to sulphur dioxide which is the actual agent for reducing the nitrogenous compounds. The nitrogen is left as ammonium sulphate, from which the am-

[1] *Bur. of. Chem., Bull.* **137,** p. 85.
[2] *Z. anal. Chem.,* **1883,** 366.

monia is liberated by potash or soda and distilled into a known excess of standard acid.

The original method of Kjeldahl has been modified at different times by the use of reagents designed to act as carriers of oxygen and thus facilitate the oxidation. Of these the two which have been most widely used are the modification of Wilfarth[1] in which mercuric oxide is used as the oxygen carrier, and the Gunning method[2] in which the boiling point of the sulphuric acid is raised by the addition of potassium sulphate. For ordinary foods either of these methods is satisfactory, but in the presence of alkaloids and certain other classes of organic compounds not all of the nitrogen is obtained.

Sherman[3] has made a study of the various methods and recommends the Dyer[4] modification in which both mercury and potassium sulphate are used. The method can be carried out as readily as either of the others and has the advantage that one method is applicable in all cases.

Digestion.—Weigh ordinarily 0.5 to 1.0 gram of sample into a 500–600 cc. Kjeldahl flask, preferably of Jena glass. Care should be taken not to get particles of the material on the neck of the flask. It can be added through a narrow trough of glazed paper or conveniently by placing the weighed sample in a small piece of clean filter paper, folding it over and introducing the whole into the flask. Add 20 cc. of pure concentrated sulphuric acid, free from nitrogen, and three small drops of mercury. Place the flask in an inclined position and close its mouth with a small glass funnel. Support the flask on wire gauze, or better on a piece of asbestos board with a hole cut in it of such a size that the flame of the burner strikes only the portion of the flask below the level of the acid. Heat with a low flame until frothing ceases, and then bring to a boil; cool slightly and add 10 grams of potassium sulphate, then heat again to brisk boiling. The operation should be conducted in a hood with a good draught.[5] The liquid should be kept boil-

[1] *Chem. Zentr.*, **1885**, 113.

[2] *Z. anal. Chem.*, **1889**, 188.

[3] *J. Am. Chem. Soc.*, **1904**, 1469.

[4] *J. Chem. Soc.*, **1895**, 811.

[5] Sy (*J. Ind. Eng. Chem.*, **1912**, 680) has described an ingenious apparatus by which the digestion can be made directly in the laboratory, the fumes of sulphuric acid being drawn from the flask and dissolved in a stream of water.

ing for at least half an hour after it has become colorless. In favorable cases the digestion may be completed within an hour, but if alkaloids or other substances that are decomposed with difficulty are present, as in pepper, the boiling should be continued for at least 3 hours.

Distillation.—After the digestion is finished allow the flask to cool somewhat, add cautiously 150 cc. of water, cool and add 20 cc. of potassium sulphide solution[1] and mix thoroughly to precipitate the mercury, which might otherwise hold back part of the ammonia as mercur-ammonium compounds. Add 100 cc. of cold saturated sodium hydroxide solution, pouring it carefully down the side of the flask so that it shall not immediately mix with the acid and liberate the ammonia. At once add about a gram of granulated zinc, which by the evolution of hydrogen prevents bumping, a piece of paraffin the size of a pea to prevent frothing, and connect the flask with the condenser. (The condenser tip should dip into a measured amount (25–40 cc.) of $\frac{N}{10}$ hydrochloric or sulphuric acid in the receiver.) Mix the contents of the flask by shaking and distil about 200 cc., taking care to rinse the delivery tube free from the acid solution at the end of the distillation. Titrate the excess of acid with $\frac{N}{10}$ alkali, using methyl orange as indicator.

Notes.—The writer much prefers to carry out the distillation by means of steam, using an apparatus as shown in Fig. 23, the time of distillation being in this way much shortened. Whatever method of distillation is employed connection between the condenser and the flask containing the alkaline solution should be made by some form of "spray trap," to prevent fine particles of alkali being carried over mechanically with the steam into the acid in the receiver.

In the steam distillation the use of zinc and paraffin is not necessary, except that the latter should be used in the case of milk. Potash can also be used in place of sodium hydroxide if desired, since the presence of the separated sulphate is not objectionable during the distillation.

The somewhat simpler Kjeldahl-Wilfarth or the Gunning

[1] Forty grams per liter.

method can be carried out in practically the same way, if desired, and for the majority of foods will give good results. The digestion, however, must be carried on for a longer time, at least 2 hours after the solution becomes colorless. Directions for these methods may be found in Woodman and Norton: Air, Water and Food; or Leach: Food Inspection and Analysis.

The reagents used in either method should of course be tested when a new lot is prepared. This is best done by making a blank determination, using 0.5 gram of cane sugar, which will

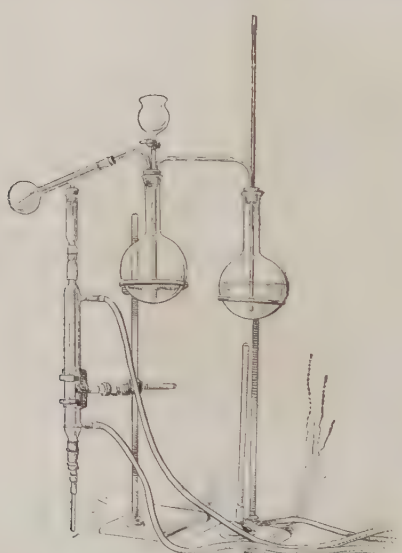


FIG. 23.—Kjeldahl distilling apparatus.

aid in the reduction of any nitrates that may be present in the reagents.

Determination of Nitrogen in the Presence of Nitrates.—Occasionally it may be required to determine total nitrogen in a sample which contains also some nitrates. By the methods just described there would be loss of nitric acid when the sulphuric acid is added for the digestion. This may be avoided by adding the acid in the presence of some substance, as phenol or salicylic acid, which is readily nitrated and thus holds the nitric acid as a nitro-derivative. The nitro-compound is then reduced and the determination carried out as before.

Method.—To the weighed sample add 30 cc. of sulphuric acid containing 2 grams of salicylic acid, pouring the acid on quickly and at once covering the sample with it. Allow it to stand with frequent shaking for 10 minutes, then add 5 grams of sodium thiosulphate, heat gently at first and then to boiling until strong fuming ceases. Add mercury and potassium sulphate and continue the method as described on page 26.

Centrifugal Methods.—An important piece of apparatus for the food laboratory is a suitable centrifuge. A comparatively simple



FIG. 24.—Electric centrifuge.

form, to be run either by hand or power, finds widespread use in the determination of fat in milk (page 113). For many purposes, however, a machine of greater capacity and capable of higher speeds is highly desirable. Such a machine, capable of carrying loads up to 1000 grams and giving a speed up to 3000 revolutions per minute, is shown in Fig. 24. One of these machines, made by the International Instrument Company of Cambridge, Mass., and embodying certain suggestions of the writer which fit it especially for the requirements of food analysis, has been in daily use for several years with entire satisfaction.

Selected References

ALLEN.—Commercial Organic Analysis, 4th Edition.
BAILEY.—Source, Chemistry and Uses of Food Products.

Beythien, Hartwich und Klimmer.—Handbuch der Nahrungsmitteluntersuchung.

Blyth.—Foods, their Composition and Analysis.

König.—Chemische Zusammensetzung der menschlichen Nahrungs- und Genussmittel.

Leach.—Food Inspection and Analysis.

Leffman and Beam.—Select Methods of Food Analysis.

Parry.—The Analysis of Food and Drugs.

Sherman.—Food Products.

Sherman.—Organic Analysis.

U. S. Dept. of Agr., Bur. of Chem., Bull. 107 (Revised). Official and Provisional Methods of Analysis.

Vaubel.—Methoden der quantitativen Bestimmung organischer Verbindungen.

CHAPTER II

THE MICROSCOPICAL EXAMINATION OF FOODS

The Value of the Microscope in Food Analysis.—With many classes of food materials, such as spices, cocoa, coffee and cereal products, the examination for adulterants is far from complete unless the sample has been examined with the microscope. This is because the microscope often reveals much more clearly than does chemical analysis the nature of the adulteration. For instance, the chemical analysis of a sample of cocoa showed that the amount of starch present was somewhat greater than usually corresponds to the other constituents, but left the analyst in doubt whether the amount was greater than could still be explained by the possible natural variation in starch content of genuine cocoa, or implied added starch. The microscope, however, in this particular case showed at a glance the presence of a distinct amount of arrowroot starch, a form which differs so markedly from the starch of the cocoa bean as to be readily distinguished from it. It would surely not be too much to say that in detecting certain forms of adulteration the microscope is the main reliance of the food analyst. If to this we add the ease and quickness with which the examination can be made, and the fact that by comparing different microscopic fields with mixtures of known composition it is often possible to approximate the percentage of the adulterant present, the great value of the microscope for this work becomes apparent, hence a certain amount of practice in its use should always accompany a course in food analysis.

Students who have had the advantage of an elementary laboratory course in plant histology will find this distinctly helpful, although the absence of such preliminary training should not deter anyone from undertaking the microscopic study of foods The microscopic structures by which the common adulterants are recognized are comparatively simple and a reasonable expenditure of time and study should enable the student to gain sufficient knowledge of the microscopical examination to supplement to great advantage the chemical analysis. To obtain the best re-

sults the work should be carried out systematically, and much time will be gained if it can be done under the direction of a competent instructor.

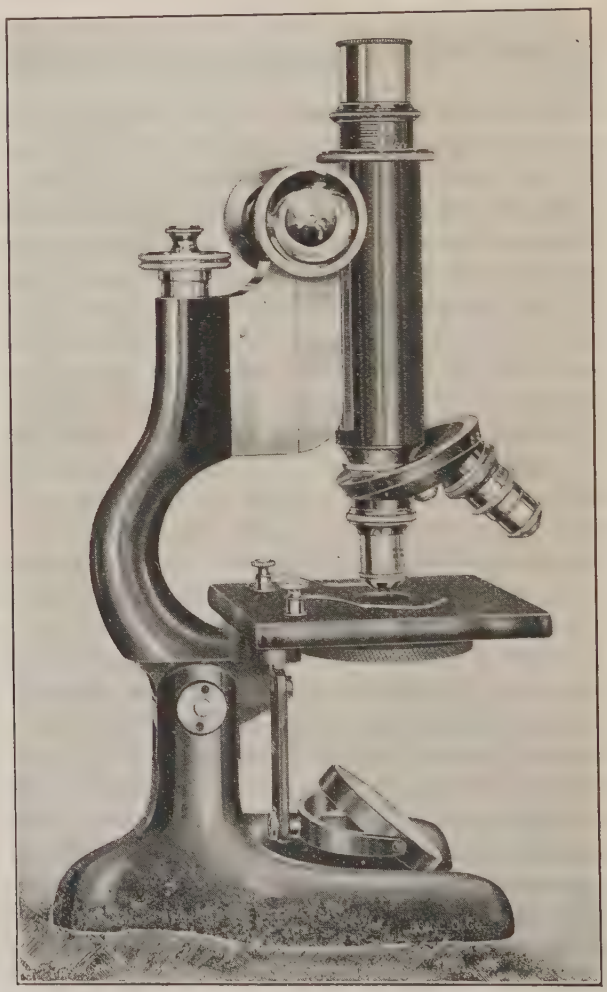


Fig. 25.—Microscope for food analysis.

Apparatus.—(*a*) *Essentials. Microscope.*—The first requisite is a suitable microscope. A thoroughly satisfactory modern instrument can be purchased for about $30.00 (Fig. 25). It

should be provided with a double nosepiece carrying two objectives of 16 and 4 mm. equivalent focus and giving with a medium eyepiece magnifications of 75 and 300 diameters. A numerical aperture of 0.65 for the higher power objective, with its accompanying greater working distance, will be found admirably adapted for food work, and the objectives, as will be the case with modern lenses, should be par-focal and accurately centered so that no time will be lost in changing from one to the other.

This, together with a suitable number of the ordinary glass microscope slides and cover glasses, the latter preferably ¾-in. circles, make up the absolute essentials of the apparatus needed.

(*b*) *Accessories.*—Of these, the most useful will be an *eyepiece micrometer* and a *micro-polariscope.* The micrometer is used to

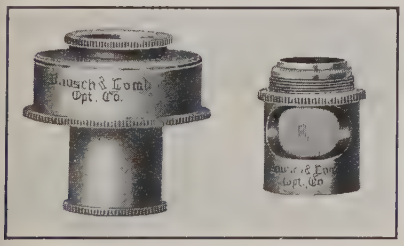


FIG. 26.—Micro-polariscope.



FIG. 27.—Abbe condenser.

measure the size of starch grains, stone-cells, etc. In its simplest form it consists of a disc of glass, bearing a scale usually graduated in 0.1 mm., which rests on the diaphragm of the eyepiece so that the scale is viewed superimposed upon the object as seen in the microscope. The exact value of the scale divisions depends upon the objective used, and can be ascertained approximately from tables in the catalogs of the principal makers or measured directly by means of a stage micrometer.

The micro-polariscope is used in the examination of starches, some of which exhibit distinct differences when viewed in this way, and in examining fats, such as butter, to tell whether or not they have been crystallized. It is an accessory which would probably find more general use if the microscope makers would provide some means of applying it quickly and conveniently. A

common form consists of a polarizer mounted so as to be used in the substage ring above the mirror, and an analyzer which may be screwed to the microscope tube above the double nosepiece or to the inner draw tube as preferred (Fig. 26).

An *Abbe condenser* (Fig. 27) with iris diaphragm attached is a decided convenience in securing sufficient illumination on dark days but it is by no means essential.

A *demonstration eyepiece*, showing a small pointer in the field, is very useful in calling attention to some definite object. One can be easily improvised by cementing a short, firm hair by means of a drop of mucilage to the diaphragm of the eyepiece in such a way that it projects nearly to the center of the field. By moving the slide it is easy to bring to the end of the pointer any

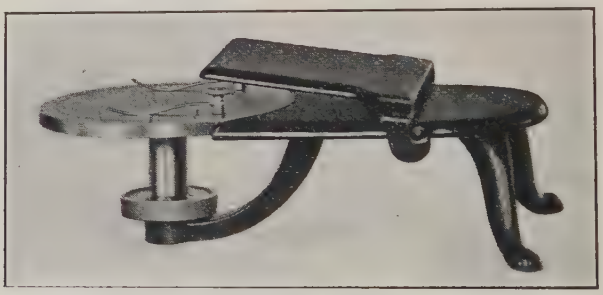


Fig. 28.—Turn table for making permanent mounts.

particular object to which it is desired to call attention. For the preparation of permanent mounts and thin sections for study a *turn table* (Fig. 28) and *sectioning razor* will be found useful.

Preparation of the Sample.—The sample will frequently, as in the case of spices, breakfast cocoa, etc., be in a sufficiently fine condition to examine directly. If quite coarse like coffee, it may be necessary to grind a small portion of it, which may be done conveniently in a small porcelain mortar. Samples like chocolate, which contain a large amount of fat or oil, may be treated several times on a small filter with portions of ether and then dried on the water-bath. The residue obtained after the treatment with sulphuric acid and alkali in the determination of crude fiber (see page 269) is often good hunting ground for stone cells and other hard tissues. A bit can be taken from the moist residue on the

point of a knife and examined under the microscope without appreciably affecting the weight.

Temporary Mounts.—The most serviceable method is to mount the sample directly in water. A bit of the powder is placed on the center of the slide, a drop of water placed near it and the powder scraped into the water with the edge of the cover glass. It is then covered with the glass and rubbed gently between the thumb and finger until the material seems to be evenly distributed and free from coarse or gritty particles. It is best not to put too much on the slide because only a confused mass will be seen through the microscope. Enough water should be used so that the cover shall lie flat on the slide, any excess being removed with a bit of filter paper.

Reagents.—For the greater part of the work the plain water mount as just described will be sufficient, but occasionally it may be desired to treat the sample with some reagent to bring out more distinctly characteristic structures, or to render the sample clearer and free from débris. The two reagents most useful are dilute iodine solution and chloral hydrate. The first of these is of great value in showing the presence of starch, especially when present in very small amount. It can be added very easily to the temporary water mount by placing a drop at the edge of the cover and touching the opposite edge with a bit of filter paper. Some of the water is withdrawn by capillary attraction and thus a portion of the iodine solution is brought into contact with the material and its action can be observed.

For dissolving the starch and other cell contents, and rendering the harder tissues more transparent for examination, the chloral hydrate may be used. A large drop of a 60 per cent. solution is placed on the slide, the powder added and covered loosely with a cover glass. The slide is then heated carefully over a small flame until the liquid boils gently, then quickly cooled by placing it on a cold surface. If necessary another drop of the solution can be drawn under the cover glass and the process repeated.

Permanent Mounts.—It is sometimes desired to mount samples of especial interest so that they shall be permanently available. This may be done by using either glycerin jelly or Canada balsam as a mounting medium instead of water.

In mounting a sample in glycerin jelly a bit of the jelly[1] is placed on a slide and warmed over a small flame until melted, a little of the powder added to it and the cover glass, previously warmed to prevent enclosure of air bubbles, gradually lowered on to the melted drop and gently pressed down. The excess of jelly which exudes can be removed when the slide is cold.

For examination by polarized light the best mountant is Canada balsam. A drop of the balsam dissolved in xylol is placed in the center of the slide, the material added and the whole covered with a warm cover glass. After standing for several days the balsam will become very hard and the excess can be scraped off with a knife.

Some of the starch grains, mounted in this way and examined with crossed Nicols exhibit characteristic and striking phenomena (see page 44).

If it is desired to preserve the mounts in glycerin jelly for any great length of time the cover glass should be sealed to prevent the jelly from drying out by evaporation. This may be done by placing a ring of cement or asphalt varnish around the cover so that it is partly on the cover and partly on the slide, using the turn table and a pointed camel's hair brush.

Authentic Samples for Comparison.—It is essential that the student should have at hand a collection of samples representing the pure spices or other products which are being examined, together with the common adulterants. A complete set, in the case of the spices for instance, would comprise a collection of the whole unground spices in order that their general characteristics may be noted and the relations of the different parts studied with a hand lens; thin sections of the various spices, cut longitudinally and transversely and mounted as permanent slides for study with the microscope, are also of great value, showing oftentimes the structures more clearly to the beginner than in the powdered sample, and giving as well the relative positions of the different tissues; finally, specimens of the powders themselves should be included, for the purpose of making temporary mounts and comparing directly with the material in question.

[1] One part of gelatin by weight is soaked in six parts of water, seven parts of glycerin are added and finally 1 per cent. of phenol. The mixture is warmed and stirred for 10 or 15 minutes until clear, and filtered hot.

Such authentic samples can be prepared without much trouble by the student himself by powdering small quantities of the whole spices, or they may be purchased, together with the prepared sections, at slight cost.[1] The laboratory should possess a syste-

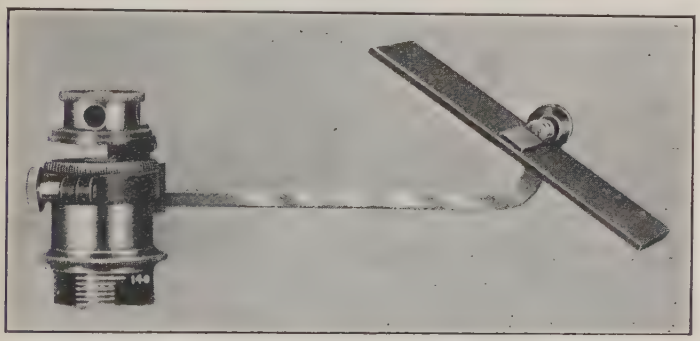


FIG. 29.—Camera lucida.

matic collection of those in most common use ready for immediate access. Known mixtures for testing the proficiency of the student at each point in his progress will also be found helpful.

Permanent Records.—If it is desired to preserve for future reference or study any particularly interesting specimen it can be drawn by means of a camera lucida (Fig. 29) or even better, photographed.

Apparatus for photomicrography can be obtained which is comparatively simple and self-contained and the process, after conditions have once been fixed, is easily and quickly carried out.

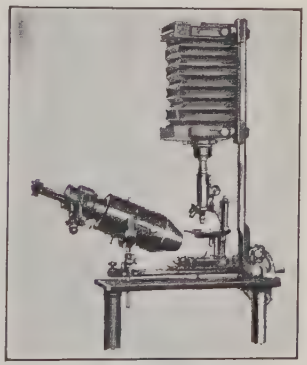


FIG. 30.—Photomicrographic apparatus.

Figure 30 shows a combined photomicrographic and drawing apparatus made by the Bausch & Lomb Optical Co. which was used by the writer with marked satisfaction in making the photomicrographs which illustrate this chapter.

[1] Eli Lilly & Co., Indianapolis, prepare such material and will send a printed list upon request.

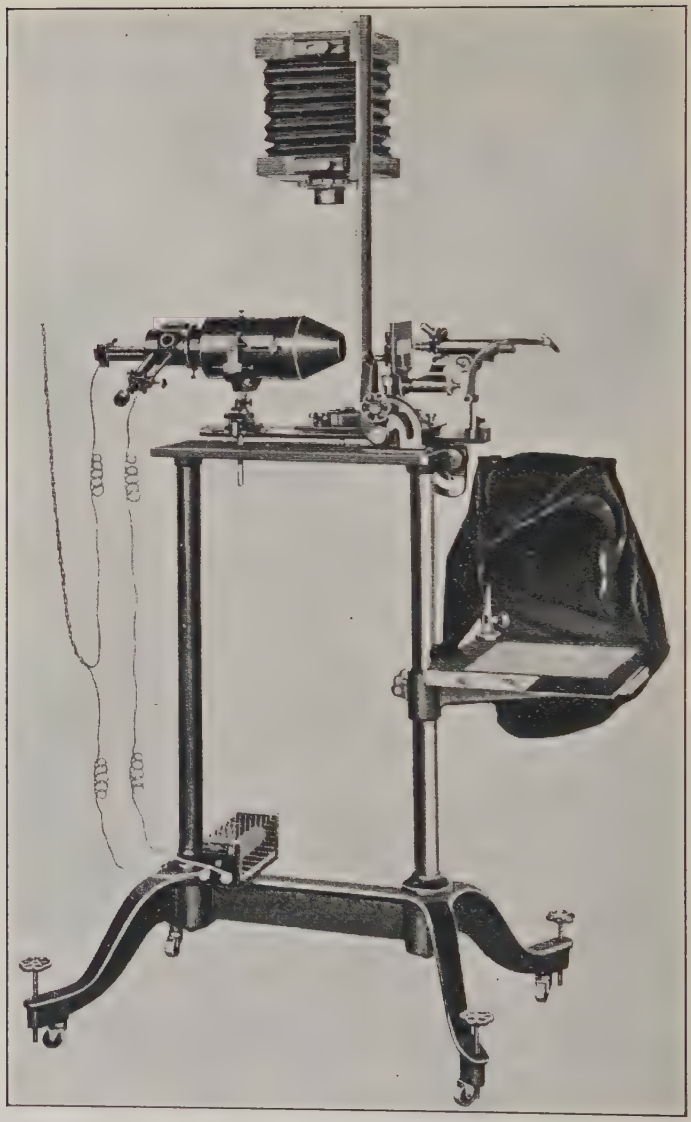


FIG. 31.—Photomicrographic apparatus arranged for drawing or demonstration.

The apparatus consists of a stand bearing two optical beds so arranged that the microscope and camera can be used in either a vertical or horizontal position, an illuminating apparatus composed of an electric lamp together with a suitable condensing system, and an adjustable drawing board. Any microscope can be used.

The vertical arrangement of the apparatus, as shown in the figure, has been found especially convenient for work with food materials because the water mount can be examined at leisure and when the desired field is found the camera can be quickly adjusted in position, the photographic plate inserted, and the exposure made. The exposure is so short, usually 2 to 6 seconds, that any movement of the particles of material can be avoided. A further distinct advantage of the photographs prepared in this way is that they can be made into lantern slides by the simple contact method and used to illustrate the subject to a large class of students.

Figure 31 shows the apparatus arranged for the projection of the image on a sheet of white paper placed on the drawing board, when the object may be readily drawn or demonstrated to a group of students. A mirror fixed in front of the eyepiece at an angle of 45° directs the image to the drawing board.

Typical Plant Tissues.—The vegetable materials used as food are often from different parts of the plant and would naturally be expected to present differing structural features under the microscope. Among the spices, for instance, ginger is a root, cassia a bark, allspice a berry, cloves are flower buds. A study of these under the microscope shows, however, that many of the plant tissues are repeated in the different parts so that the number that are of principal importance is not so great; as a matter of fact, the features which serve as guides or marks of identification to the food microscopist are comparatively few in number and quite easily recognized.

The most important of them are:

Parenchyma.—This is the name given to the thin-walled cellular structure of the softer portions of the plant. The cells are usually more or less regular in outline and may or may not still show the cell contents. Typical examples are shown at p_1, p_2 and p_3 in Fig. 32.

Stone Cells.—These occur most frequently in the protective portions of the plant, in the shells of nuts, in the bark, and in the coatings designed to protect the delicate seeds. They are irregular in shape and differing in color, but have in general thickened walls and very prominent radial cracks extending through the walls. With polarized light and crossed Nicols they show a brilliant play of colors. (See Figs. 33 and 34 for characteristic forms.)

Bast Fibers.—Bast fibers are of the same general nature as stone cells, but differing in that they are very long in proportion to their width, with tapering pointed ends, and a very narrow central canal. They are most commonly found in the fibro-vascular elements or sap-conducting tissues of the plant. Distinctive forms of these are to be seen in Fig. 35.

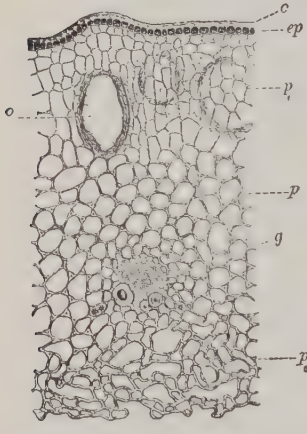


Fig. 32.—Typical parenchyma, p_1, p_2, p_2. (Moeller.)

Ducts and Vessels.—The fibro-vascular bundles contain also various forms of vessels or ducts, which are thin-walled tubes variously known according to the appearance of the thickenings in the walls as *annular*, *spiral*, *scalariform* or *reticulated* ducts. In the softer portion of the bundle occur *sieve tubes*, peculiar thin-walled cells with porous partitions known as *sieve plates*. (See Fig. 36 and Fig. 105*a*, page 502.)

Cell Contents.—Of the important features of the cell contents two should be noted here—*starch* and *resin*. The starch is, upon the whole, the chief reliance of the food microscopist in the identification of the various adulterants on account of the wide differences in its size and shape, which will be discussed in greater detail below.

The resins, from their striking color, are at times of great service in indicating the presence of adulterants. Typical examples are allspice and Bombay mace.

The appearance of these characteristic structures can be shown very clearly in prepared sections of the plant. Viewed in this

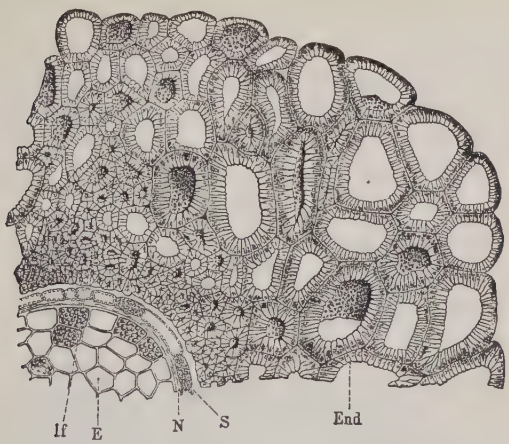


FIG. 33.—Stone cells, *End.* (WINTON.)



FIG. 34.—Stone cells from the shell of the cocoanut. (WINTON.)



FIG. 35.—Typical bast fibers. (VOGL.)

way they can be pointed out to the student more readily than when a powder, consisting of a more or less confused mass of disintegrated tissues, is examined under the microscope. A list of such prepared sections which the writer has found helpful is:

Parenchyma.—Elder pith (transverse and longitudinal sections) coffee.

Stone Cells.—Pepper, pericarp of allspice.

Bast Fibers.—Clove stems, cassia (longitudinal sections).

Vessels and Ducts.—Chicory, ginger, *Pteris*, squash.

Starch in situ.—Wheat, bean.

Resins.—Bombay mace.

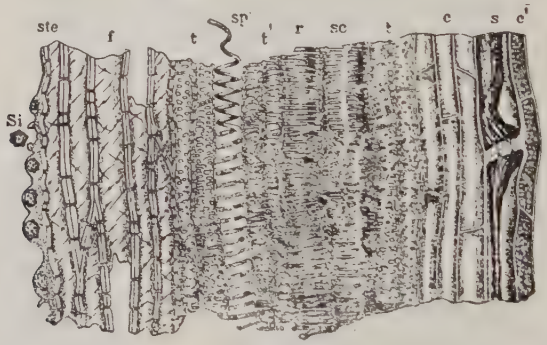


FIG. 36.—Fibro-vascular bundle from the mesocarp of the cocoanut; *t* tracheids with small pits; *t'* tracheids with large pits; *sp* spiral vessel; *r*, reticulated vessel; *sc* scalariform vessel; *s* sieve tube. (WINTON.)

Starches.—The ability to differentiate and recognize under the microscope the various starches is of great importance. Water mounts of the pure starches should be made and studied until the student can recognize any in a mixture. The points to be noted are, especially, size, shape, markings, groupings, presence or absence of hilum and its position in the granule. Examination by polarized light, noting the presence of polarization crosses with dark field, and a play of colors with a selenite plate, may also be helpful. Care should be taken to choose a portion of the slide where the grains are not crowded too closely together, and a preparation mounted in balsam should be used for examination by polarized light.

It should be borne in mind in examining the starches that not all of the grains in a given microscopic field will be of the exact

size or shape that are characteristic of a particular starch. For instance, wheat starch is characterized as a circular starch with a central hilum. In a given field there will be some grains that are oval or lens-shaped and that show no hilum. Such individual cases should be neglected and attention paid to the majority of the granules. It is true also that accidental contamination may be found with one or a few grains of a different starch. Such should be disregarded and those taken into consideration which appear to be present in the greatest proportion and in every field.

Possibly the simplest classification of the starches is that depending upon their general shape into circular, oval, elliptical and polygonal. The common ones would be divided under this method into:

Circular.—Wheat, rye, barley, tapioca.

Oval.—Potato, arrowroot, sago.

Elliptical.—Pea, bean.

Polygonal.—Corn, oats, buckwheat, rice.

(*a*) **Circular Starches.**—*Wheat Starch.*—The grains are irregularly circular in outline, or where they are tipped up on edge they may appear lens-shaped. There are two principal sizes of granules, the larger, which are more characteristic, varying in size from 30-40μ[1] and the more numerous smaller ones, averaging about 5μ. The larger grains usually show a small central dotted hilum, and at times concentric rings, especially by oblique light. The appearance by polarized light is not especially characteristic. (Fig. 61, page 495.)

Rye Starch.—This starch is of the same general character as wheat starch, comprising two sizes of granules. The larger grains are somewhat greater in size than the corresponding grains of wheat, a considerable proportion being over 50μ in diameter. The concentric rings are rather more prominent and the hilum frequently shows slight cracks radiating from it. (Fig. 52, page 495.)

Barley Starch.—The granules of barley starch closely resemble those of wheat but are somewhat smaller, seldom being over 35μ in diameter. (Fig. 63, page 495.)

[1] The unit of measurement in microscopical work is the *micron*, denoted by the Greek letter μ, and occupying one-thousandth of a millimeter (0.001 mm.).

Tapioca Starch.—This starch is smaller than those that have just been described, averaging about 15–20μ, the grains being fairly uniform in size. They are in general quite round and are characterized by many of the grains being truncated or shaped like a kettle-drum. The hilum is usually a distinct central dot, and the rings are not ordinarily seen. (Fig. 64, page 495.)

(*b*) **Oval Group.**—*Potato Starch.*—This is a typical oval starch and the largest of the common starches. The grains average 60–80μ in diameter with very pronounced rings, the so-called "oyster-shell markings." The hilum is a dot at the smaller end of the granule. With crossed Nicols the polarization crosses are very showy, and with a selenite plate a brilliant play of colors is observed. (Figs. 65 and 66, page 495.)

Arrowroot Starch.—There are several varieties of starch known under the name of arrowroot, but the term is here limited to the West India product. This starch resembles potato in a general way, but the grains are somewhat smaller, seldom over 50μ, and the hilum is usually a transverse fissure at the larger end of the granule. It often shows two wings, as in the conventional sign for a bird flying. The rings are quite distinct and the polarization effects very pronounced. (Fig. 67, page 496.)

Sago Starch.—This is another of the larger starches, the grains averaging 40μ in size and individuals being occasionally 80μ in length. In shape they are irregularly oval, the larger ones often showing one or more protuberances with flattened surfaces, indicating contact with other grains in aggregates which have been broken up in the process of manufacture. The hilum is toward one side of the granule and is frequently cracked. The rings are distinct, and characteristic crosses are shown by polarized light. (Fig. 68, page 496.)

(*c*) **Elliptical Group.**—*Pea Starch.*—The starches of the pea and the bean are very similar and typical of the leguminous starches in general. They are ellipsoidal in shape, averaging about 50μ in length, with distinct rings. The hilum is usually a pronounced cleft with numerous lateral branches. The polarization crosses are $\asymp$ shaped. (Fig. 70, page 496.) The bean starch is shorter and more rounded, as well as smaller than the pea. (Figs. 69 and 71, page 496.)

(*d*) **Polygonal Starches.**—*Corn Starch.*—This is the largest and most common of the polygonal starches, some grains being 30μ in diameter, though 20–25μ is more usual. The grains sometimes occur in groups of three or more, but more commonly singly. The hilum is centrally placed and often shows radiating cracks. (Fig. 72, page 496.)

Buckwheat Starch.—This starch is somewhat similar to corn but is not so sharply angular, is somewhat smaller, ranging in size from 6–12μ, and the hilum is less pronounced and does not show the radiating cracks. Very characteristic, however, is the grouping into polygonal masses. Both the individual starch grains and the aggregates are shown in Fig. 73, page 497.

Oat Starch.—The oat starch belongs also to the polygonal group. The granules are comparable in size with those of buckwheat, although somewhat smaller, seldom exceeding 10μ in diameter. Like buckwheat, too, they form aggregates, which are usually round or elliptical in shape. Occasional grains are long and spindle shaped, which is fairly characteristic of this starch. (Fig. 74, page 497.)

Rice Starch.—Rice is the smallest of the common polygonal starches, the grains averaging about 5μ in diameter. Masses of grains occur sparingly, not nearly so commonly as in buckwheat or oat starch. The individual grains are quite sharply angular, this and the size serving to distinguish them from the other members of the polygonal group. The hilum and rings are seldom seen. (Fig. 75, page 497.)

Typical Food Products.—The examples given in the following pages have been selected as being well fitted to give the student an idea of the forms of adulteration to be detected by the microscope, and to show its value. The list is by no means complete and no attempt has been made to make it so. Some of the spices, as allspice and ginger, are included for their value in microscopical training although not discussed chemically. The special advantage of the synopsis which is given for each product is that the beginner's attention is focused upon the tissues of prime importance from the analyst's standpoint, and he is not distracted by the minor details which are very properly given in the descriptions and figures which aim to show complete microscopical structure.

The proper procedure in each case is for the student, having become familiar with the prominent plant tissues through the examination of typical sections, and studied the starches in water mounts, to examine then in systematic order powdered samples of the pure spice in question and its common adulterants, noting the important features as summarized in the synopsis. His proficiency should then be tested by examining and reporting upon several prepared mixtures whose composition is unknown to the student. Having done this with a fair degree of accuracy, he is ready to examine the unknown sample with confidence.

The list of adulterants given in each case does not include everything that could possibly be used for adulteration, but things that have been variously reported as actually found. Reference should constantly be made to the authorities given in the list at the end of this chapter, especially to the works of Winton and Leach.

Allspice.—(Fig. 76, page 497.)

CHARACTERISTICS.

Starch.—Small (av. diam. = 8μ), nearly circular, uniform in size, central dotted hilum. (Fig. 76, *a*.)

Stone Cells.—Large, colorless, plainly marked and quite numerous. (Fig. 76, *b*.)

Resin.—Yellow, brown or red lumps of waxy luster and striking appearance. Especially characteristic. (Fig. 76, *c*.)

ADULTERANTS.

Clove Stems.—(Fig. 77, page 497.) (See also Fig. 88, page 499.)

Stone cells, very similar to those of allspice; bast fibers, allspice has none (Fig. 88, *a*.); vascular ducts, especially characteristic. (Fig. 77, *a*; Fig. 88, *b*.)

Nut Shells.—(Fig. 78, page 497.)

Stone cells are usually long and spindle shaped, yellow brown in color with brown contents; bits of colorless trachea. (Fig. 78, *a*.)

Fruit Stones.—(Fig. 79, page 498.)

Masses of long colorless stone cells resembling those of cocoanut shells but free from color.

Cayenne.—(Fig. 80, page 498.)

Outer skin of fruit pod, usually reddish brown in color with a

cellular structure (*a*); epidermis of seed is greenish yellow with very peculiar and characteristic markings resembling the convolutions of the intestines (*b*).

Pepper.—For the characteristic structures of pepper in greater detail see page 50. It is most readily recognized in mixtures by the characteristic polygonal masses of starch grains. (Fig. 101, page 501.)

Turmeric.—See Mustard, page 50.

Ginger.—(Fig. 93, page 500). (See also page 49.)

Starch, oval, smooth, showing neither hilum nor rings, but many granules have characteristic small protuberances at one end, as at *a*, *a*, Fig. 97, page 501.

Pea Hulls.—(Figs. 81 and 82, page 498.)

Pea starch (page 44) accompanied by aggregates of the long rectangular cells of the palisade layer, averaging 60–100μ in length. (Fig. 82, *a*.)

Cereals.—Recognized by the characteristic starches, page 43, *et seq.*

Cassia.—(Cinnamon).—(Fig. 83, page 498.)

CHARACTERISTICS.

Wood fibers, red brown in color, often grouped in bundles with grains of starch interspersed. (Fig. 83, *a*.)

Bast Fibers.—(Fig. 83, *b*.)

Stone cells, resembling somewhat those of allspice, but brownish in color, rather more oblong and usually with one wall distinctly thicker than the other. (Fig. 83, *c*.)

Starch.—The starch is quite similar to that of allspice, but often occurs as compound grains made up of two to four single grains. These may at first glance appear to be large single grains, but close inspection will show their compound nature.

Yellow patches of cellular tissue with starch grains showing through may be abundant.

ADULTERANTS.

Ground Bark.—The fibers from this resemble the woody fibers of cassia, but are coarser and usually do not show the starch grains interspersed that characterize the cassia tissues. (Fig. 82, *a*, page 498.)

Ginger.—Recognized best by the starch. (See page 49.)

Sawdust.—The long coarse fibers occur in bundles often crossed at right angles by the medullary rays (Fig. 85, *a*, page 499.) Sometimes the fibers or tracheids show distinct openings or pores.

Turmeric.—See Mustard, page 50.

Cassia Buds.—These have many tissues in common with cassia but may be distinguished by broader, shorter bast fibers and especially by short, thick-walled crooked hairs. (Fig. 86, *a*, page 499.)

Cereals.—Distinguished by the characteristic starches.

Cloves.

CHARACTERISTICS.—(Fig. 87, page 499.)

Confused mass of cellular tissue; few bast fibers.

ADULTERANTS.

Clove Stems.—(Fig. 88, page 499, and 77, page 497.) Recognized by characteristic vascular ducts and stone cells as given under allspice.

Nut Shells.—See Allspice, page 46.

Fruit Stones.—See Allspice, page 46.

Allspice.—The element best suited for showing allspice is the colored resin. (Fig. 76, *c*, page 497, and 89, *a*, page 499.)

Ginger.—See Allspice, page 46.

Cereals.—Recognized by their characteristic starches, page 43, *et seq.*

Cocoa.

CHARACTERISTICS.—(Fig. 90, page 499.)

Starch.—Small, nearly circular, hilum central, dotted, rather indistinct; frequently occurs in twins or triplets. (Fig. 90, *a*.)

Pigment Cells.—Yellow, brown or violet in color.

Dark miscellaneous débris of the cotyledons.

ADULTERANTS.

Cocoa Shells.—(Fig. 91, page 500.) *Spiral ducts* may occur in long spirals (*a*), or simply as fragments showing only as a ring or half a link of a chain (*b*). Occasional fragments occur in the pure ground cocoa, on account of the difficulty of separating the shells completely in the process of manu-

facture, but their presence in distinct and recurring amounts is indicative of the presence of shells.

Cereals, Etc.—Wheat, arrowroot, sago, corn. Distinguished by the characteristic starches, which are usually much larger than those of cocoa and easily recognized. (See Fig. 92, page 500.)

Ginger.

CHARACTERISTICS.

Starch.—(Fig. 93, page 500.) Oval, flattened, often with a tapering point at the smaller end which serves to distinguish it from wheat, which it most nearly resembles. The average size is 25 μ. The hilum and rings are indistinct and not of much assistance in identification. The starch is the most prominent feature of powdered ginger.

Scalariform Vessels.—(Fig. 94, *a*, page 500.) Occasional wood fiber with starch grains showing through (Fig. 94, *b*).

ADULTERANTS.

Turmeric.—See Mustard, page 50.

Cayenne.—See Allspice, page 46, also Fig. 95, *a*, page 500.

Sawdust.—See Cassia, page 48. Note the distinction between the natural fibers of the ginger and the coarser tracheids of ordinary sawdust, as shown at *a* and *b* in Fig. 96, page 500.

Cereals.—Characteristic starches. Fig. 9, page 501, shows ginger adulterated with wheat and corn.

Mustard.

CHARACTERISTICS.—(Fig. 98, page 501.)

General appearance is that of a confused mass of gray cellular tissues. No starch is present.

Mustard hulls, shown by bits of the yellow and brown palisade cell layer (Fig. 98, *a*) may appear here and there in the pure powdered mustard.

Epidermal Cells (Fig 98, *b*).—Colorless, cellular and mucilaginous.

ADULTERANTS.

Mustard Hulls.—The presence of the characteristic palisade layer in abnormal amounts, at times even exceeding the proportion of the regular tissues of the seed, or in large

masses as in Fig. 99, page 501, is an indication of the admixture of hulls. Comparison should be made with the amount found in powdered genuine mustard flour.

Turmeric.—The bright yellow color of the "paste balls" is very characteristic. The starch is oval, large with very distinct "oyster shell" markings, but fragile and easily broken up, so not of so great importance for identification. (Fig. 100, *b*, page 501.)

Cereals.—Identified by their characteristic starches, as at *a*, Fig. 100.

Pepper.

CHARACTERISTICS.—(Fig. 101, page 501, see also page 340.)

Starch.—Individual grains are very small, averaging about 3μ in diameter. They are polygonal in shape and show a distinct central hilum. Of more importance for identification are the aggregates or polygonal masses of closely packed grains. (Fig. 101, *a*.)

Stone Cells.—These are yellow, very thick walled, with a tendency toward square ends. (Fig. 102, *a*, page 501.)

Other elements, of less importance, but which occur occasionally, are groups of the cup-shaped "beaker cells," bits of the brown or yellow parenchyma of the shell and needle-like crystals of piperin. (Fig. 103, page 502.)

ADULTERANTS.

Added Pepper Shells.—This is usually determined microscopically by the presence of an excessive number of stone cells of the shell. Comparison should be made with the number to be noted in several whole pepper corns ground in a porcelain mortar. (Fig. 102, *a*, page 501.)

Olive Stones.—See Allspice, page 46.

Nut Shells.—See Allspice, sage 46.

Turmeric.—See Mustard, page 50.

Long Pepper.—Characteristic disagreeable odor, more pronounced when heated. With polarized light and crossed Nicols the starch masses show a glimmering white appearance quite different from that of true pepper. The appearance is a little more striking if the amount is made in glycerin.

Buckwheat.—Distinguished by the greater size of the starch aggregates and of the individual grains. (Fig. 104, *a* and *b*, page 502.)

Other Cereals.—Distinguished by their starches, page 43, *et seq.*

Selected References

GREENISH.—Microscopical Examination of Food and Drugs.
LEACH.—Food Inspection and Analysis.
NELSON.—Introduction to the Analysis of Drugs and Medicines.
SCHIMPER. — Mikroskopischen Untersuchungen der Nahrungs- und Genussmittel.
TSCHIRCH AND OESTERLE.—Anatomischer Atlas der Pharmakognosie und Nahrungsmittelkunde.
VILLIERS AND COLLIN.—Traité des altérations et falsifications des substances alimentaires.
VOGL.—Die wichtigsten vegetabilischen Nahrungs- und Genussmittel.
WINTON.—Microscopy of Vegetable Foods.

CHAPTER III

FOOD COLORS AND PRESERVATIVES

Colors

The addition of coloring matter of food products, apart from any question of the harmful nature of the color itself, may be objectionable or otherwise, depending upon the character of the food to which it is added and the object desired in the addition.

If the color is added simply to satisfy the æsthetic sense of the consumer and does not in any way constitute a deception, there can be no serious objection to its use. In candy, for instance, the consumer is perfectly aware that the color is entirely artificial, and if the color used is harmless the practice is unobjectionable.

In other cases, however, color is added to conceal inferiority or to simulate an appearance of greater value. Such practises as the addition of yellow color to pastry to imitate the presence of eggs, or the injection of red color into watermelons to give the appearance of ripeness, are obviously fraudulent. In the majority of such cases the interests of the purchaser are sufficiently protected if the package is plainly marked to show the presence of artificial color. It is obvious that the use of color which is in any way harmful should not be permitted under any circumstances.

Kinds of Color Used.—The coloring matters employed in foods may be conveniently divided into three classes: Coal-tar dyes, vegetable colors and mineral colors. Of these the coal-tar colors are used to a much greater extent than the others.

Mineral Colors.—The class of mineral colors may include pigments used directly, as oxide of iron (red ocher) in coloring anchovy and similar fish pastes, and ultramarine for coloring sugar; or mineral compounds of colors, as the so-called *lakes*. These are insoluble compounds of vegetable colors or of coal-tar dyes, usually with a metallic base, as aluminum or tin.

The mineral colors, being insoluble, are usually readily seen as colored particles when a portion of the food material is exam-

ined under the microscope and their presence may be confirmed by testing the ash for such metals as iron, tin, aluminum, lead, chromium or antimony. Minute traces of iron or aluminum should be ignored, since they occur naturally in the ash of many food materials. Some of the lakes, especially of cochineal and logwood, are used quite extensively in confectionery, hence if added color is suspected and the ordinary tests for coal-tar dyes or vegetable color are negative, the possibility of a lake being present should be considered. In such cases, the lake may be decomposed with a dilute solution of hydrochloric acid and the color either dyed on wool or extracted by amyl alcohol and tested as described under the other classes of colors.

Vegetable Colors.—The colors of animal or vegetable origin which are most likely to be found in foods are comparatively few, the use of such colors having greatly decreased owing to the advantages of the coal-tar dyes. The following are those of chief importance: Annatto, archil, caramel, chlorophyll, cochineal, cudbear, logwood, Persian berries, saffron and turmeric.

Separation of Vegetable Colors.—All of these colors, except caramel and chlorophyll, may be extracted from a solution or suspension of the food material by acidifying with hydrochloric acid and shaking out in a separatory funnel with amyl alcohol. The extract obtained in this way, however, may contain fat, sugars and other portions of the food which would interfere with subsequent tests for the color, so it must be carefully purified. To do this, wash the amyl alcohol layer twice with small quantities of water, and evaporate the amyl alcohol on the water-bath. Take up the residue in 50 per cent. alcohol, filter, and shake twice with petroleum ether to remove fat. Draw off the alcohol layer, dilute with an equal volume of water, add a few drops of hydrochloric acid and shake out again with amyl alcohol. This amyl alcohol may be washed once with water to remove the excess of acid and the fairly pure color obtained by evaporation on the water-bath.

To identify the color, it may be dissolved in water and tested as in Table IV.[1] The reduction test with zinc and hydrochloric acid is conveniently made in a test-tube, using about 0.2 gram of zinc dust and 10 drops of strong acid. The test with sulphuric acid

[1] Loomis: *Bur. of Chem., Circular* **63.**

TABLE IV.—REACTIONS OF COLORS IN AQUEOUS SOLUTION AND WITH CONCENTRATED SULPHURIC ACID

Name of color	Color of aqueous solution	Add to aqueous solution			Zn dust + HCl and exposure to air on filter paper	Dry color + conc. H_2SO_4	
		HCl (1.12) 5–10 drops	10 per cent. NaOH 5–10 drops	NH_4OH (0.96) 5–10 drops		Before dilution	After dilution
Annatto	Yellow in alkaline solution	Paler	.....	.....	.....	Blue	Mauve
Archil (Cudbear)	Deep lilac	Yellowish pink	Purple	Purple	Orange; restored	Purple	Red to orange
Cochineal	Orange red	Orange yellow	Magenta	Magenta	Orange yellow; not restored	Pink	Yellow pink then straw yellow
Logwood	Yellowish brown	Orange	Dark brown	Light brown	Color not restored	Yellow brown	Paler
Persian berries	Yellow	No change	Orange	Deeper	Not decolorized	Yellow	Paler
Saffron	Yellow	No change	Paler	No change	Color not restored	Blue, purple, maroon, red brown	Yellow, then nearly colorless
Turmeric	Yellow in alkaline solution	Paler	.....	.....	With Zn and NaOH not decolorized	Orange	Dirty yellow

TABLE V.—REACTIONS OF VEGETABLE COLORS ON WOOL, MORDANTED WITH TIN

Color	Hydrochloric acid (sp. gr. 1.16)	Sulphuric acid (sp. gr. 1.84)	Nitric acid	Stannous chloride, hydrochloric acid and water	10 per cent. sodium hydroxide	Boiling alcohol
Annatto	Unchanged	Olive green	Lemon yellow	Unchanged	Fiber and solution bluish purple; color slowly removed	Bluish red solution
Archil and Cudbear	Solution and fiber red	Fiber and solution purple; on dilution red, fiber almost colorless	Yellow	Decolorized	Orange	.....
Cochineal	Orange red	Dark violet	Yellow	Orange	Red violet	No color
Logwood	Red violet	Olive brown, yellow on dilution	.....	Red to violet	Violet	No color
Persian berries	Little affected	Yellow olive	.....	Fiber yellower	.....	.....
Saffron	Darker	Olive green, then red brown; fades on dilution	.....	Not decolorized	Darker	.....
Turmeric	Fiber reddish, solution pale pink; on dilution fiber bright yellow	Fiber and solution reddish brown; on dilution fiber pale straw, solution colorless	At first deep red, then yellow	Bright reddish orange fiber and solution	.....	Yellow solution, green fluorescence

on the dry color may be made on small amounts of color by streaking the acid with a glass rod across a spot of the evaporated solution in a porcelain dish. The reactions should be observed in as concentrated a solution of the color as can be obtained.

A portion of the solution should also be acidified with a drop of dilute hydrochloric acid and evaporated nearly to dryness with a piece of wool previously mordanted with tin.[1] The dyed wool is washed and dried, then portions of it tested with reagents as in Table V, page 55.[2]

If necessary, the special tests described on pages 56 to 58 may be used in confirmation. It should be remembered also that many of the natural fruit colors dissolve in amyl alcohol, giving a colored solution, and the analyst should be assured that the reactions obtained are not due to the fruit color. (See *Bur. of Chem., Bull.* **107** (rev.), p. 193, and *J. Pharm. Chim.*, **1901**, 174.)

It is always best to confirm any conclusions by tests made on an actual sample of the color in question.

Special Tests for Vegetable Colors.—*Archil* and *Cudbear.*—These two lichen colors dye wool in the same manner as the coal-tar dyes and hence are included in that section (pages 58 to 86).

Caramel.—Practically the only positive test for small amounts of caramel is the precipitate which it gives with paraldehyde.[3] The test is carried out by adding to the clear color solution, concentrated to small bulk, three times its volume of paraldehyde, then adding alcohol in small portions with frequent shaking until the mixture is just homogeneous. If caramel is present, a brown adherent precipitate will form on standing (over night in the case of small amounts).

To use this test successfully, several precautions must be observed. Care should be taken in concentrating the solution of color that the heat employed is not sufficient to caramelize any sugar that may be present. The temperature should not rise above 70°C. or so. In adding the alcohol only enough should be added so that the aqueous solution and the paraldehyde do not

[1] In 500 cc. of water dissolve 0.8 gram of tin crystals and 0.4 gram of oxalic acid. Boil 10 grams of fat-free wool 1½ hours in this solution. Wring and dry at room temperature. Keep in a stoppered bottle away from the light.

[2] Berry: *Bur. of Chem., Circular* **25.**

[3] Amthor: *Z. anal. Chem.*, **1885**, 30.

separate on standing for several minutes, not necessarily enough to make the mixture entirely free from turbidity. If considerable caramel is present, the liquid will be turbid and precipitation may commence as soon as the liquids have been mixed. In the case of materials containing much sugar or gum, paraldehyde may cause a precipitate resembling caramel. In such cases it is advisable to separate the caramel by a preliminary precipitation, conveniently with zinc hydroxide, which exercises a somewhat selective action, dragging down the caramel to a greater extent than the gum and sugar.[1]

To 10–20 cc. of the practically neutral solution to be tested, add 2 cc. of zinc chloride (5 per cent. solution) and 2 cc. of potassium hydroxide (2 per cent. solution). The precipitate is filtered, washed with hot water and dissolved in 15 cc. of 10 per cent. acetic acid. The acid solution is concentrated to about a third of its volume, the excess of acid nearly neutralized, the solution filtered, if not perfectly clear, and precipitated with paraldehyde as described above.

Other tests are sometimes employed to show the presence of caramel in foods, but they are for the most part negative tests, depending on analytical differences between the caramel and the natural color of the particular food and are not of general application. Such tests are described under Vanilla and Whiskey, pages 388 and 485.

Cochineal.—If a portion of the amyl alcohol solution of the color is shaken with dilute ammonia, a purple color is produced in the presence of cochineal. A still more characteristic test is given with uranium acetate. If the amyl alcohol solution is shaken with water and a 3 per cent. solution of uranium acetate added drop by drop, with frequent shaking, the aqueous layer becomes a deep emerald green.

Saffron.—Saffron is most likely to be encountered in such products as macaroni, noodles and pastry. The best solvent for extracting it is 70 per cent. alcohol made slightly ammoniacal (3 drops of 0.96 ammonia to 100 cc. of 70 per cent. alcohol). The coarsely ground material is digested at room temperature for several hours with the solvent, which is then filtered off. The acid coal-tar dyes, like Naphthol Yellow S, which are added to

[1] Woodman and Newhall: *Tech. Quart.*, **1908**, 280.

macaroni are also readily dissolved by this treatment and can be dyed on wool and identified as directed under coal-tar colors.

The 70 per cent. alcohol extracts also the "lutein" or yellow coloring matter of the flour, as well as color from the eggs, if any are present. These can be removed, however, by evaporating the alcoholic solution to dryness on the water-bath and extracting the residue with ether, in which the saffron and most of the coal-tar colors that are used are nearly insoluble, while the natural color dissolves. If preferred, the original material may be extracted with ether before removing the artificial color with the 70 per cent. alcohol. A mixture of 7½ parts of acetone to 1 of water may also be used for the preliminary extraction. The saffron may be taken out from the aqueous solution left after evaporating the alcohol by acidifying with hydrochloric acid and extracting with amyl alcohol. It is best to purify the color as described on page 53 after which the purified residue may be tested for saffron by drawing across it a glass rod moistened with strong sulphuric acid, which gives an immediate, but very fugitive blue color. A similar color is given by concentrated nitric acid. It is essential that the color be as pure as possible, since otherwise the charring with the acid will produce red or purple colors which mask the saffron reaction. The blue color of the saffron test appears *immediately*, changing quickly to red and finally to brown.

Turmeric.—This is readily detected by the well-known boric acid test. An alcoholic extract of the material is evaporated almost to dryness on the water-bath with a piece of filter paper and a few drops of a saturated solution of boric acid. If turmeric is present, the dried paper will be a cherry-red color, which is changed to bluish green by a drop of sodium hydroxide or ammonia.

Coal-tar Colors.—Although the number of coal-tar dyes is very great, Mulliken[1] listing some 1500 individuals, the number that are used in food products is comparatively limited. On account of the use to which they are put in foods, they are for the most part readily soluble in water, and fortunately for the analyst, most of them are *substantive*, dyeing directly on wool without requiring the use of a mordant. The greater number are "acid" dyes, that is, they will dye wool from a faintly acid solution, while a few are

[1] Identification of Pure Organic Compounds.

"basic" dyes, being best taken up by the wool from a slightly alkaline bath.

The following list includes, with the exception of a few oil-soluble colors, practically all the coal-tar dyes that have met with any application in coloring foods. The numbers at the left of the table refer to Schultz and Julius' standard list of dyes[1] and the names in parentheses are common synonyms for the same dye.

1. Picric Acid.
3. Naphthol Yellow (Martius Yellow, Manchester Yellow).
4. Naphthol Yellow S (Martius Yellow S, Acid Yellow S).
8. Fast Yellow G (Fast Yellow, Acid Yellow).
13. Croceine Orange (Brilliant Orange, Ponceau 4GB).
14. Orange G.
43. Orange GT (Crocein Orange Y).
53. Palatine Scarlet (Brilliant Cochineal 2R).
55. Ponceau 2R (Xylidine Red, Xylidine Scarlet).
56. Ponceau 3R (Cumidine Red, Cumidine Ponceau).
65. Fast Red B (Bordeaux B).
71. Azoeosine.
84. Resorcin Yellow (Tropaeolin Yellow, Chrysoine).
85. Orange I (Naphthol Orange).
86. Orange II (Gold Orange, Mandarin G).
87. Orange III (Methyl Orange, Helianthine).
88. Orange IV (Tropaeolin 00, Diphenylamine Orange).
89. Brilliant Yellow S.
94. Tartrazine.
95. Metanil Yellow.
102. Fast Red A.
103. Azorubin S (Fast Red C, Carmoisin).
106. Brilliant Scarlet (New Coccin, Cochineal Red A).
107. Amaranth (Fast Red D).
108. Ponceau 6R (Scarlet 6R).
160. Croceine Scarlet 3B (Ponceau 4RB).
163. Biebrich Scarlet (Fast Ponceau B, Ponceau 3RB).
169. Croceine Scarlet 7B.
240. Congo (Congo Red).
269. Chrysamine R (Chrysamin).
277. Benzopurpurine 4B.
278. Benzopurpurine 6B.
287. Azo Blue.
398. Naphthol Green B.
425. Auramine.
427. Malachite Green (New Green, Fast Green).

[1]A Systematic Survey of the Organic Coloring Matters. Edited by A. G. Green, 1904.

428. Brilliant Green (Ethyl Green).
435. Light Green SF Yellowish (Acid Green).
439. Cyanol Extra (Acid Blue 6G).
440. Patent Blue VN (New Patent Blue B, 4B).
448. Magenta (Fuchsine, Aniline Red).
451. Methyl Violet B (Methyl Violet DB).
452. Crystal Violet.
462. Acid Magenta (Acid Fuchsine).
464. Acid Violet 4BN.
468. Formyl Violet 4BS (Acid Violet 4B extra).
476. Methyl Alkali Blue.
480. Soluble Blue (Water Blue).
504. Rhodamine B.
510. Uranine (Fluorescein).
512. Eosine (Eosine A).
517. Erythrosine.
518. Phloxine P.
520. Rose Bengal.
523. Rose Bengal 3B.
584. Safranine.
585. Methylene Violet 2RA.
601. Induline Soluble (Fast Blues).
650. Methylene Blue (Methylene Blue B, NG).
667. Quinoline Yellow (Chinoline Yellow).
692. Indigo carmine (Indigo disulpho-acid).

Permitted Dyes.—With a view to restricting the coal-tar colors used in foods to those whose harmlessness is beyond question, it is forbidden under the Federal Food and Drugs Act[1] to use any coal-tar dyes except the seven following: Amaranth (107), Ponceau 3R (56), Erythrosine (517), Orange I (85), Naphthol Yellow S (4), Light Green SF Yellowish (435), and Indigo disulpho-acid (692). These may be used under definite regulation as to their purity and mode of manufacture.[2]

Identification of Coal-tar Colors.—In the identification of a coal-tar color in food two methods are commonly employed to isolate the color: Dyeing on wool and extraction with immiscible solvents.

Dyeing on Wool.—*Procedure.*—If the material is a liquid, use about 50 cc. directly; if a solid, about 25 grams should be mixed with water as thoroughly as possible and made up to a volume of

[1] U. S. Dept. Agr., Food Inspection Decision 76.
[2] U. S. Dept. Agr., Food Inspection Decision 77.
(See also *Bur. of Chem., Bull.* **147.**)

approximately 100 cc. If the solution is not already acid, add a drop of hydrochloric acid (sp. gr. 1.12) or enough to produce a very slight but distinct acid reaction. Add a piece of white woolen cloth about 2 in. square, which has been thoroughly washed in boiling water, and boil in the colored solution for at least 10 minutes, replacing the water lost by evaporation. Remove the wool and if colored rinse thoroughly in boiling water to remove any color which may be adherent to the fiber. Strip the color from the wool by boiling with dilute ammonia (1 part of strong ammonia to 50 parts of water). Remove the wool, add dilute hydrochloric acid to the solution until it is faintly acid, immerse a fresh piece of the woolen cloth and boil again for 10 minutes. In general, a distinct color on the second piece of wool indicates the presence of a coal-tar dye.

Notes and Precautions.—It is not necessary to have a perfectly clear solution of a solid material, although it should not be so thick as to bump badly when boiled.

The size of the piece of cloth used for dyeing should be governed somewhat by the amount of color present. If only a trace of color is evident, less wool should be employed in order that it shall be as deeply dyed as possible. This applies perhaps more strongly to the second dyeing than to the first, since the color can be collected from the original solution on a fairly large piece of wool and then concentrated on a smaller piece in the second dyeing.

Care should be taken that the dye bath is not too strongly acid. Some of the colors, Naphthol Yellow S for example, do not dye well in a strongly acid bath and hence might be overlooked. Enough only should be added to give a slight but distinct reddening to blue litmus paper. In many cases, as in fruit products, the food itself will contain sufficient organic acid without adding the hydrochloric acid. When only small amounts of color are present it is often best to obtain a fairly clear solution and evaporate nearly to dryness on the water-bath with a trace of acid and a small piece of wool. For the subsequent identification of the dye a piece of wool only an inch square, dyed to a full shade, is better than a much larger piece merely tinged with color.

An essential point is to watch the progress of the dyeing in the first bath. It is best to use a succession of rather small pieces of cloth, removing one as soon as it seems to have taken up what

color it will, then adding another, and so on until the color is exhausted from the solution. In this way it may usually be determined whether more than one color is present, the different dyes often dyeing the wool at unequal rates. The actual separation of the colors may be carried out in the same way, although larger amounts can usually be obtained for identification more readily by separation with immiscible solvents as described on page 63.

If the only purpose is to detect the presence of a coal-tar dye, the single dyeing and stripping outlined above will ordinarily suffice. If, however, the dyed wool is to be used for identifying the color, especially if vegetable color is also present, a further stripping and dyeing is advisable in order that the color may be as pure as possible.

While the dyeing method is the simplest and in many respects the best method for detecting coal-tar colors, its limitations should be borne in mind. The method as described is not suited for basic dyes, and if these are suspected the method should be reversed, the dyeing being done in weak ammonia solution (1 part of strong ammonia to 50 of water) and the stripping made by dilute acetic acid (5c c. of glacial acid to 100 cc. of water). Basic dyes are less commonly found in foods than are the acid colors.

Archil and cudbear dye wool by this procedure in a manner similar to the coal-tar colors but can be recognized readily by the purple color of the wool when stripping with the ammonia, and by the fact that the dyed fiber is decolorized by zinc and hydrochloric acid, the color being restored on exposure to the air. (See also page 55.) Indigo carmine is changed by stripping with ammonia, hence may not be found in the second dyeing. If there appears to be a blue color present which dyes the first piece of wool but not the second, a piece of the first dyeing should be tested for indigo as on page 73.

The vegetable colors (except archil and cudbear) give practically no color in the second dyeing by this test, although if very large amounts are present some may persist, and it may be necessary to strip and re-dye several times. The presence of a vegetable color can frequently be predicted from the change in color of the wool when treated with ammonia.

Extraction with Solvents.—Although not of so general application as the dyeing test described above, extraction with solvents is sometimes employed when somewhat larger quantities of color are to be separated for identification. The most suitable solvent is amyl alcohol and the method may be outlined as follows:

Prepare an aqueous solution of the colored material by macerating and straining or filtering if necessary, make it alkaline with sodium hydroxide and shake with amyl alcohol in a separatory funnel, carefully avoiding the formation of an emulsion. Test a little of the amyl alcohol layer for basic colors by shaking it with dilute acetic acid in a test tube. If the lower layer is colored, a basic or weakly acid dye may be present and the main portion of the amyl alcohol should be evaporated to dryness on a water-bath, adding a little ethyl alcohol from time to time to hasten the evaporation. Take up the residue in hot water and dye the color on wool from a bath faintly ammoniacal in the case of basic dyes, and acid with a drop of acetic acid with weakly acid dyes.

The alkaline solution from which the basic colors have been removed is made strongly acid with one-half its volume of hydrochloric acid (sp. gr. 1.20) and again shaken with amyl alcohol and 2 or 3 cc. of ethyl alcohol. Most of the acid colors will be dissolved by the amyl alcohol, although it may not be deeply colored itself. A few of the highly sulphonated colors will still remain in the aqueous layer. The amyl alcohol is drawn off and shaken with dilute sodium hydroxide to remove the color. The aqueous layer is separated, acidified and the color dyed on wool as described on page 60. If the amyl alcohol still shows some color, it can usually be removed by adding an equal volume of petroleum ether and again shaking with sodium hydroxide. It should be borne in mind that the amyl alcohol will dissolve vegetable colors, so that the presence of coal-tar dyes should not be assumed unless shown by a double dyeing of the color extracted by the amyl alcohol.

Identification of Coal-tar Dyes.—Although the identification of a coal-tar color is much more difficult than its simple detection in a food, the examination of a product for color can hardly be considered complete unless this be done.

The first essential is to obtain from the material by the methods just mentioned as large a quantity of the dye, and in as pure a condition as possible, both dyed on wool and in aqueous solution. It may be possible to do this very easily, as in the case of confectionery, in which the color is frequently in the form of a surface coating, readily removed by a slight washing with water. With other foods the color may be extracted by a suitable solvent and obtained pure by several washings and re-extractions. If the most available method of separating the color seems to be by dyeing on wool, a solution for testing may be obtained by stripping a portion of the dyed wool with dilute ammonia, evaporating the ammoniacal solution to dryness on the water-bath and taking up the residue in a little water.

The color may be identified by testing either the dyed wool or the aqueous solution and it is usually best to use both methods. The tests should not be applied haphazard but in a definite order.

Identification by Tests on the Fiber

As regards systematic tests on the fiber, the procedure recommended by Green, Yeoman and Jones[1] has in the writer's experience proven most useful.

The following reagents are used:

Ammonia (1:100).—1 cc. of ammonia (sp. gr. 0.90) to 100 cc. of water.

Acetic acid (5 per cent.)—5 cc. of glacial acetic acid to 95 cc. of water.

Hydrosulphite A.—Use a freshly prepared 10 per cent. solution of "Rongalite."[2]

Hydrosulphite B.—Acidify 200 cc. of Hydrosulphite A with 1 cc. of glacial acetic acid.

Persulphate.—A cold saturated solution of potassium persulphate.

Procedure.—The tests are carried out in test tubes, using bits of the dyed cloth about ¼ in. square and 5 cc. of the reagent. In judging the degree of "stripping" compare the amount of color remaining on the cloth with that of the origi-

[1] *J. Soc. Dyers* and *Colourists*, **1905**, 236.

[2] Rongalite C (Sodium formaldehyde-sulphoxylate, $CH_2\langle^{OH}_{O.SONa} + 2H_2O$) is a powerful reducing agent prepared and sold by the Badische Anilin-u. Sodafabrik. The solution slowly decomposes on standing and should not be used when more than a few days old.

nal, rather than with the amount of color in solution. In the tests with Rongalite, the wool is boiled for 15 to 60 seconds with the reagent, the latter poured off, the cloth thoroughly rinsed and allowed to lie on white paper for an hour. If the color does not return, the same piece is boiled in a test tube with 5 cc. of water, and persulphate added drop by drop, avoiding an excess. If this also fails to cause any return of color, the dye belongs to the *azo* group.

Only the yellow, orange, and red shades of acid colors from Green's tables are given here, these being the most commonly occurring shades in foods. For the complete tables reference may be made to the original paper, to *Allen's Commercial Organic Analysis, 4th Ed., Vol. V*, or to the *Journal of the Society of Chemical Industry*, **1905**, 1034.

TABLE VI.—GREEN'S SYSTEMATIC SCHEME FOR THE IDENTIFICATION OF DYES ON THE FIBER

A. Yellow and Orange Colors.

Boil twice for 1 minute with dilute ammonia (1:100) and a piece of whit cotton. Keep the ammoniacal extract.

I. Much color is stripped but cotton remains white.

Acid Dye. Boil with hydrosulphite B.

1. Color is unaffected. QUINOLINE or PYRONE GROUP. (Quinoline Yellow, Uranine, Eosin Orange).

2. Decolorized and color does not return on exposure to air or with persulphate. AZO or NITRO GROUP.

Add concentrated HCl to ammoniacal extract.

(*a*) No change of color. (Tartrazin, Orange G, Orange 2G, Orange R, etc.)

(*b*) Becomes colorless. (Martius Yellow, Naphthol Yellow S).

(*c*) Becomes red. (Fast Yellow, Indian Yellow, Azoflavine, etc.)

(*d*) Becomes violet or violet red. (Metanil Yellow, Orange IV).

II. Little or no color is stripped. *Salt or Mordant Dye.* Consult complete table (*loc. cit.*).

B. Red Colors.

Boil twice for 1 minute with dilute ammonia (1:100) and piece of white cotton. K ep the ammoniacal extract.

I. Much color is stripped but cotton remains white—*Acid Dye.* Boil with hydrosulphite B.

1. Color of wool not altered. PYRONE GROUP.

Acidify the ammoniacal extract.

(*a*) Precipitation and disappearance of fluorescence. (Eosin, Phloxine, Erythosine, etc.)

(*b*) No precipitation, and fluorescence remains. (An acid Eosin or acid Rhodamine).

2. Decolorized. Color returns on exposure to air. AZINE GROUP. (Azo Carmin or Rosinduline).
3. Decolorized. Color does not return on exposure to the air, but restored by persulphate. TRIPHENYLMETHANE GROUP. (Acid Magenta.)
4. Decolorized. Color not restored by air or by persulphate. AZO GROUP.

Boil with dilute bichromate.

Color unaffected. Evaporate ammoniacal extract and treat with conc. H_2SO_4.

(*a*) Red solution. (Xylidine or Palatine Scarlet.)
(*b*) Violet solution. (Crystal Scarlet, Fast Red A, etc.)
(*c*) Blue solution. (Crocein Scarlet, Fast Red B, etc.)
(*d*) Green to dark maroon or violet black. (Chromotropes, Azofuchsin, etc.)

II. Some of the color is stripped and the wool becomes much bluer. Boil with hydrosulphite A.

1. Color slowly changed to deep yellow. Original color not restored on exposure to air. (Cochineal Scarlet.)
2. Decolorized. Color is quickly restored on exposure to air. (Archil.)

IDENTIFICATION BY TESTS ON THE AQUEOUS SOLUTION

If a small quantity of an aqueous solution of the color, of a reasonable degree of purity, can be obtained, it is oftentimes easier to identify the dye by the systematic application of color reactions and solubility tests to the solution. The tables in ordinary use for this purpose, such as those of Weingärtner,[1] or Rota[2] will not be found of much value in food work since they require in many cases considerably larger quantities of color than can usually be obtained from a food product. Of much greater assistance is the table of solubilities and the detailed scheme proposed by Loomis.[3] Even this is based on the behavior of 0.01 per cent. solutions of the dyes, a distinctly stronger solution than is available in many cases, so that with more dilute solutions the reactions described are not always

[1] *J. Soc. Dyers and Colourists*, **1887**, 67.
[2] *Chem.-Ztg.*, **1898**, 437.
[3] *U. S. Dept. Agr., Bur. of Chem., Circular* **63.**

characteristic. In the table given below[1] the tests have been planned to identify colors which are present in concentrations of 0.01, 0.002, and 0.001 per cent. The primary division into blue and violet, red, yellow and orange, and green colors is based on the color dyed on wool in a faintly acid bath. This simple scheme does not differentiate the similar dyes Ponceau 2R and Ponceau 3R; Orange III and Metanil Yellow.

TABLE VII.—ANALYTICAL SCHEME FOR IDENTIFICATION OF COAL-TAR DYES BY TESTS MAINLY ON THE AQUEOUS SOLUTION

In adding the reagents such as NaOH and HCl, a drop is added at a time. Usually the first drop gives some results. In trying any test on the dyed fiber, a piece of the dyed cloth is wet with distilled water and used for comparison so that a brightening of the color due to moisture will not be ascribed to the action of the reagents. In most cases the reactions on the dyed fiber are not used as a means of differentiating the dyes, since good sharp color distinctions are obtained only with the deeply dyed fiber, while the reactions with NaOH, HCl and concentrated H_2SO_4 also hold for the more dilute solutions.

Unless stated otherwise, all tests are made on small portions (2 to 5 cc.) of the aqueous solution. This aqueous solution may be obtained by extracting the color with amyl alcohol, as on page 63, evaporating and dissolving in water, or, in the case of most colors, by stripping the dyed wool with ammonia, evaporating to dryness on the water-bath to expel ammonia, and dissolving in a little water.

Tests on the "dry dye" are made by evaporating 2 to 5 cc. of the aqueous solution in a small porcelain dish, adding 10 drops of strong sulphuric acid and rubbing it over the dye with a glass rod. After noting the color, 10 cc. of water is added and any change in color observed.

If in using the scheme a dye is reached which does not agree in its confirmatory tests with the unknown sample, the procedure should be carefully traced back to make sure that an error in judgment at some point has not placed the unknown in the wrong group.

A. Blue and Violet Dyes

Add conc. H_2SO_4 to dry dye

1. BLUE.—Add 10 per cent. NaOH to aqueous solution.

Crimson	Azo Blue (287)
Green	Indigo Carmine (692)

2. RED.—

Aqueous solution blue	Methyl Alkali Blue (476)
Aqueous solution violet	Soluble Blue (480)

[1] Miss Hattie D. F. Haub, Thesis, Mass. Inst. of Technology, 1912.

3. Pale Yellow or Colorless.—Treat dyed fiber with 10 per cent. NaOH.

Greenish yellow to yellow	Cyanol Extra (439)
Bluish green to green	Patent Blue VN (440)

4. Purple or Violet.—Add 10 per cent. NaOH to aqueous solution.

Violet	Induline Soluble (601)
Green	Indigo Carmine (692)

5. Green.—

Aqueous solution red violet, no change on dilution	Methylene Violet 2RA (585)
Aqueous solution blue, greenish blue on dilution	Methylene Blue AD (650)

6. Orange.—To 5 cc. of aqueous solution, add 1.5 cc. 10 per cent. NaOH and 5 cc. of ether and shake 30 seconds. Separate water completely from ether and wash latter twice with 2 cc. of water + 2 drops of 10 per cent. NaOH, separating water completely each time. Finally, shake ether with 2 cc. of water and 1 cc. of 25 per cent. acetic acid.

1. Color of acetic acid approximately equal to original color. Add 10 per cent. NaOH (1 drop to each 2 cc.) to aqueous solution and shake with ether.

Ether straw yellow	Methyl Violet (451)
Ether colorless	Crystal Violet (452)

2. Color of acetic acid only slight fraction of original color. Compare color of very dilute aqueous solution with known dyes.

Blue violet	Acid Violet 4BN (464)
Red violet	Formyl Violet, 4BS (468)

B. Red Dyes

I. Dilute aqueous solution strongly fluorescent. Shake aqueous solution acidified with HCl (1 drop of 1.12 acid for every 2 cc.), with an equal volume of ethyl acetate.

A. Extract has pink fluorescence. Rhodamine B (504)

B. Extract colorless.

Add concentrated H_2SO_4 to dry dye.

1. Becomes orange, colorless on dilution. Phloxine P (518).
2. Becomes yellow, orange red on dilution. Eosine (512).

II. Dilute aqueous solution not fluorescent. (In 0.01 per cent. solution Erythrosin shows faint fluorescence which decreases with dilution.)

Add concentrated H_2SO_4 to dry dye.

A. Crimson or red.

Shake aqueous solution acidified with HCl (1 drop of 1.12 acid for every 2 cc.), with ethyl acetate.

(*a*) Much color extracted.	Azoeosine (71).
(*b*) Almost no color extracted.	Palatine Scarlet (53).
	Xylidine Red (55).
	Ponceau 3R (56).

Distinguish by careful tests on dyed fiber and comparison with known colors.

B. Purple or violet.

Shake aqueous solution acidified with HCl (1 drop of 1.12 acid for every 2 cc.), with amyl alcohol.

(*a*) Color nearly all extracted.

Treat dyed fiber with concentrated HCl.

(*aa*) Blue — Crocein Scarlet 3B (160).

(*bb*) Red

Treat dyed fiber with NH_4OH.

(1) Violet — Archil (710).

(2) Pink — Fast Red A (102). Azorubin S (103).

(*b*) Almost no color extracted.

(*aa*) Dry dye with concentrated H_2SO_4 gives *red* violet. Brilliant Scarlet (106).

(*bb*) Dry dye with concentrated H_2SO_4 gives *blue* violet.

1. Color of dry dye is violet. Amaranth (107).
2. Color of dry dye is red brown. Ponceau 6R (108).

C. Blue.

Add HCl to the aqueous solution.

(*a*) Becomes blue. — Congo Red (240).

(*b*) Becomes purple. — Benzopurpurine 4B (277). Benzopurpurine 6B (278).

(*c*) Practically unchanged.

To aqueous solution add one-fifth vol. of 10 per cent. NaOH and shake with ether.

1. Extracted. — Safranine (584).
2. Not extracted.

(1) Dyed fiber violet red, deepened by concentrated HCl. Bordeaux B (65)

(2) Dyed fiber orange red, greenish blue with concentrated HCl. Crocein Scarlet 7B (169).

D. Orange.

Add 10 per cent. NaOH to aqueous solution.

a. Decolorized.

Shake aqueous solution acidified with HCl (1 drop of 1.12 acid for every 2 cc.), with amyl alcohol.

(*aa*) Color in upper layer. — Magenta (448).

(*bb*) Color in lower layer. — Acid magenta (462).

b. No change. — Erythrosine (517). Rose Bengal (520, 523).

Compare carefully with known samples. Rose Bengal is pink in aqueous solution, Erythrosine is red orange. If enough pure color is available test for Cl and I.

E. Green. — Biebrich Scarlet (163).

C. Yellow and Orange Dyes

I. Aqueous solution has intense green fluorescence.
Color all extracted by acidified amyl alcohol. Uranine (510).

II. Aqueous solution not fluorescent.
Add conc. H_2SO_4 to dry dye.

1. COLORLESS.
Add HCl to aqueous solution and shake with ethyl acetate.
All extracted. Picric acid (1)
Practically none extracted. Auramine (425).

2. ORANGE OR YELLOW.

A. Yellow.
Add HCl to aqueous solution.
(*a*) No change. Tartrazine (94).
(*b*) Decolorized.
Shake with ethyl acetate, separate from aqueous layer, wash once with a little water and shake with dilute NH_4OH.
(*aa*) NH_4OH colored yellow. Naphthol Yellow (3)
(*bb*) NH_4OH colorless. Naphthol Yellow S (4).

B. Orange.
Add HCl to aqueous solution.
(*a*) Becomes orange. Fast Yellow G (8).
(*b*) No change.
Add NaOH to aqueous solution.
(*aa*) No change.
Dry color and dyed wool orange. Orange GT (43).
Dry color and dyed wool yellow. Quinoline Yellow (667).
(*bb*) Becomes redder.
Dyed wool is yellow. Resorcin Yellow (84).
Dyed wool is orange red. Crocein Orange (13).

3. CRIMSON OR RED.
a. Crimson. Orange G (14).
b. Orange red. Orange GT (43)

4. PURPLE OR VIOLET.

A. Red violet.
Add HCl (1.12) to aqueous solution and shake with ethyl acetate.
(*a*) Much color extracted. Orange II (86).
(*b*) No color extracted. Brilliant Yellow S (89).

B. Blue violet.
Add HCl (1.12) to aqueous solution.
(*a*) Decolorized. Chrysamine R (269).
(*b*) Not decolorized.
Add NaOH to aqueous solution.
(*aa*) Becomes brown red. Orange I (85).
(*bb*) No change. Orange III (87).

Orange IV (88).
Metanil Yellow (95).
(In 0.01 per cent. solution NaOH gives a white ppt. with Orange III on standing.)

D. Green Dyes

Shake color solution acidified with HCl and saturated with NaCl with acetone.

A. Color nearly all extracted.
Add 2 drops HCl (1.12) to 5 cc. dilute aqueous solution.

Green.	Malachite Green (427).
Yellow.	Brilliant Green (428).

B. No color extracted.
Add 10 per cent. NaOH to aqueous solution.

Decolorized.	Light Green SF (435).
No change.	Naphthol Green B (398).

The identification of a color by the systematic tests described in Table VII should not be regarded as conclusive until it has been confirmed by tests on the dyed fiber as described in the following table (Table VIII).

The tests are carried out, using the four reagents named, on small pieces of the dyed cloth on a porcelain tile or in small white porcelain dishes. The colors are noted after the reagent has acted for about 30 seconds. Three results are given in each case, for the different intensities of color obtained with the dye solutions as described on page 67. The abbreviation in parenthesis is for the firm from which the dye was obtained.[1] The number directly preceding the dye refers, as on page 59, to the Schultz and Julius tables.

The reactions given in Table IX on page 81, if they have not been used in the analytical scheme, should also be tried.

An excellent plan, and one always to be followed in doubtful cases, is to dye a piece of wool with a genuine sample of each of the doubtful colors, taking care to have the intensity of the dyed fabric about equal that of the sample dyed from the food product, and then to apply the reagents in directly comparable tests to all the samples.

[1] A. D.: American Dyewood and Ext. Co.
B.: Badische Co.
Ber.: Berlin Aniline Works.
N.A.: National Aniline and Chemical Co.
S.H.H.: Schoelkopf, Hartford, and Hanna.

Collections of samples of the colors likely to be met in foods may be obtained from Eimer and Amend, New York.

TABLE VIII.—COLOR REACTIONS OF DYED FIBER

A. Violet and Blue Colors.

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(287) *Azo Blue (Ber.):*				
0.01	No change	Blue	Red	Red violet
0.002	No change	Greenish blue	Pink	Pink violet
0.001	Partly decolor.	Dirty green black	Decolor.	Decolor.
(439) *CyanolExtra(Ber.):*				
0.01	Yellow	Greenish yellow	Green	No change
0.002	Faint yellow	.....	Decolor.	No change
0.001	Decolor.	.....	Decolor.	No change
(440) *Patent Blue VN (A.D.):*				
0.01	Yellow	Green yellow	Greener	No change
0.002	Pale yellow	Yellow	Greener	No change
0.001	Decolor.	Pale yellow	Greener	No change
(451) *Methyl Violet B (B.):*				
0.01	Brownish yellow	Brownish yellow	N. decolor.	Paler
0.002	Faint brown	Pale brown	Decolor.	Decolor.
0.001	Decolor.	Brown	Decolor.	Decolor.
(452) *Crystal Violet (B.):*				
0.01	Orange	Orange	Partly decolor.	Partly decolor.
0.002	Pale yellow	Yellow	Decolor.	Decolor.
0.001	N. decolor.	Brownish	Decolor.	Decolor.
(464) *Acid Violet 4BN (B.):*				
0.01	Deep yellow	Orange	N. decolor.	Much fainter
0.002	Yellow	Yellow	Decolor.	Much fainter
0.001	Decolor.	Faint yellow	Decolor.	N. decolor.
(468) *Formyl Violet 4BS (B.):*				
0.01	Yellow	Deep orange	N. decolor.	Lighter
0.002	Pale yellow	Orange	Decolor.	Lighter
0.001	Decolor.	Yellow	Decolor.	Lighter

TABLE VIII.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

A. Violet and Blue Colors.

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(476) *Methyl Alkali Blue (S.H.H.):*				
0.01	Greenish blue	Red brown	N. decolor.	Lighter
0.002	Faint greenish blue	Red brown	Decolor.	Lighter
0.001	Faint greenish blue	Red brown	Decolor.	Lighter
(480) *Soluble Blue (S.H.H.):*				
0.01	Bluer	Red brown	Red brown	Decolor.
0.002	Greenish brown	Brown	Decolor.	Decolor.
0.001	Greenish blue	Brown	Decolor.	Decolor.
(585) *Methylene Violet 2 RA (S.H.H.):*				
0.01	Pale blue	Pale green	Slightly fainter	No change
0.002	N. decolor.	Greenish yellow	Slightly fainter	No change
0.001	Decolor.	Brownish	N. decolor.	No change
(601) *Induline Soluble (A.D.):*				
0.01	Fainter	Poor violet	Violet	B. violet
0.002	Fainter	Brownish	N. decolor.	Decolor.
0.001	Fainter	Brownish	Decolor.	Decolor.
(650) *Methylene Blue (A.D.):*				
0.01	Blue green	Bright green	Paler	No change
0.002	N. decolor.	Green	N. decolor.	No change
0.001	Decolor.	Yellow green	Decolor.	No change
(692) *Indigo Carmine (S.H.H.):*				
0.01	More bluish	Dark blue	Greenish yellow	No change
0.002	N. decolor.	Blue	Yellow	N. decolor.
0.001	Decolor.	Yellow	Faint yellow	Decolor.

TABLE VIII.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

B. Reds

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(53) *Palatine Scarlet (B.):*				
0.01	Crimson	Magenta	Yellow brown	No change
0.002	Pinker	Pink violet	Light brown	No change
0.001	Slightly pinker	Brownish	Light yellow	Slightly decolor.
(55) *Xylidine Red (Ber.):*				
0.01	Crimson	More crimson	Orange	No change
0.002	Pinker	Orange pink	Brown	No change
0.001	Pinker	Orange pink	.....	.....
0.0004	.....	Brown	Decolor.	.....
(56) *Ponceau 3R (Ber.):*				
0.01	Deeper pink	Scarlet	Red orange	No change
0.002	Deeper pink	Slightly more orange	Red orange	No change
0.0004	Slightly pinker	Brownish	Decolor.	Slightly decolor.
(65) *Bordeaux B (Ber.):*				
0.01	Violet	Blue	Red brown	No change
0 002	More violet	Greenish blue	Almost decolor.	Slightly decolor.
0.001	Almost no change	Dirty greenish	Decolor.	Slightly decolor.
(71) *Azoeosine:*				
0 01	Red violet	Dark red violet	Orange	Red orange
0.002	Faintly more violet	Dark brown	N. decolor.	Dirty salmon
0.001	Partly decolor.	Dark brown	Decolor.	N. decolor.
(102) *Fast Red A (A.D.):*				
0.01	Violet red	Blue violet	Dark red	No change
0.002	Violet red	Poor violet	Orange pink	No change
0.001	Light red	Poor violet	Orange pink	No change
(103) *Azorubine S (B.):*				
0.01	Deepens slightly	Blue violet	Orange red	Redder
0.002	Deepens slightly	Dirty violet	N. decolor.	Decolor.
0.001	No change	Light brown	Decolor.	Decolor.

TABLE VIII.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

B. Reds

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(106) *Brilliant Scarlet (A.D.):*				
0.01	Crimson	Violet	Brown	No change
0.002	Pinker	Poor lavender	Yellow brown turns paler	No change
0.001	No change	Dirty brownish	Faint yellow deepens on standing	Partly decolor.
(107) *Amaranth (B.):*				
0.01	Violet red	Violet	Red brown	Slightly decolor.
0.002	No change	Dirty violet	N. decolor.	Decolor.
0.001	No change	Light brown	Decolor.	Decolor.
(108) *Ponceau 6R (B.):*				
0.01	Crimson	Dark violet	Brown	No change
0.002	No change	Brownish violet	Yellow brown	No change
0.001	No change	Brown	Faint yellow	No change
(160) *Croceine Scarlet 3B (A.D.):*				
0.01	Dark blue	Magenta	Poor violet brown	No change
0.002	Blue	Violet red	Brown	No change
0.0004	Light blue	Violet brown	Light brown	No change
(163) *Biebrich Scarlet (B.):*				
0.01	Dark blue	Dark green	Deep violet	No change
0.002	Blue	Green	Violet	Decolor. more yellow.
0.0004	Decolor.	Faint yellow	Decolor.	Decolor.
(169) *Croceine Scarlet 7B (Ber.):*				
0.01	Greenish blue	Greenish blue	More violet	No change
0.002	Light greenish blue	Dirty brownish blue	N. decolor. brown pink	No change
0.001	N. decolor.	Brown	N. decolor.	No change
(240) *Congo (Ber.):*				
0.01	Dark blue	Very dark blue	More orange	More orange
0.002	Blue	Dirty blue	More orange	More orange
0.001	Light blue	Greenish blue	Slightly decolor.	Slightly decolor.

TABLE VIII.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

B. Reds

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(277) *Benzopurpurine 4B (A.D.):*				
0.01	Greenish blue	Dark blue	More orange	More orange
0.002	Blue	Blue	Orange pink	No change
0.001	Light blue	Dirty blue	Faint orange pink	Partly decolor.
(278) *Benzopurpurine 6B (A.D.):*				
0.01	Blue	Dark blue	More orange	More orange
0.002	Bluish green	Bluish green	More orange	More orange
0.001	Bluish green	Bluish green	More orange	N. decolor.
(448) *Magenta (S.H.H.):*				
0.01	Yellow brown	Brown	N. decolor.	N. decolor.
0.002	Decolor.	Pale brown	Decolor.	Decolor.
0.001	Decolor.	Light brown	Decolor.	Decolor.
(462) *Acid Magenta (S.H.H.):*				
0.01	N. decolor.	Brownish yellow	Decolor.	Decolor.
0.002	N. decolor.	Brownish yellow	Decolor.	Decolor.
0.001	N. decolor.	Brownish yellow	Decolor.	Decolor.
(504) *Rhodamine B (Ber.):*				
0.01	Pink orange	Poor yellow	Bluer	No change
0.002	Decolor.	Poor yellow	N. decolor.	No change
0.001	Decolor.	Poor yellow	N. decolor.	Decolor.
(512) *Eosine (S.H.H.):*				
0.01	Orange	Orange	No change	No change
0.002	Yellow	Yellow	No change	No change
0.001	Pale yellow	Pale yellow	No change	No change
(517) *Erythrosine (A.D.):*				
0.01	Deep orange	Orange	No change	No change
0.002	Orange yellow	Orange	No change	No change
0.001	Pale yellow	Yellow	No change	No change
(518) *Phloxine P (B.):*				
0.01	Yellow	Deep orange yellow	No change	Pink
0.002	Pale yellow	Yellow orange	No change	Pink
0.001	Decolor.	Yellow	Slightly decolor.	Pink

TABLE VIII.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

B. Reds

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(520) *Rose Bengal (Ber.):*				
0.01	Yellow	Orange	Slightly deeper	No change
0.002	Decolor.	Light brown	No change	No change
0.001	Decolor.	Light brown	No change	No change
(523) *Rose Bengal 3B (B.):*				
0.01	Red orange	Red orange	No change	No change
0.002	Decolor.	Yellow	No change	No change
0.001	Decolor.	Pale yellow	No change	No change
(584) *Safranine (SHH):*				
0.01	Greenish blue	Green	Slightly redder	No change
0.002	Decolor.	Light brown	Decolor.	No change
0.001	Decolor.	Light brown	Decolor.	N. decolor.
(710) *Archil (B.):*				
0.01	Scarlet	Brown	Violet	Violet
0.002	Pink	Light brown	Violet	Violet
0.001	Pink	Yellow brown	Violet	Violet

C. Yellows and Oranges

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(1) *Picric Acid (Ber.):*				
0.01	Decolor.	N. decolor.	Orange	No change
0.002	Decolor.	N. decolor.	Orange	No change
0.001	Decolor.	N. decolor.	N. decolor.	No change
(3) *Martius Yellow (AD):*				
0.01	Decolor.	Paler	No change	No change
0.002	Decolor.	Paler	No change	No change
0.001	Decolor.	Paler	No change	No change
(4) *Naphthol Yellow S (B):*				
0.01	Decolor.	N. decolor.	Paler	Paler
0.002	Decolor.	N. decolor.	Paler	Paler
0.001	Decolor.	Decolor.	Paler	Paler

TABLE VIII.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

C. Yellows and Oranges

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(8) *Fast Yellow B (B):*				
0.01	Red	Darker	No change	No change
0.002	Pink	Darker	No change	No change
0.001	Pink	Darker	No change	No change
(13) *Croceine Orange (Ber.):*				
0.01	Orange red	Deeper orange	Brownish orange	No change
0.002	Pink	Deeper orange	N. decolor.	No change
0.001	Pinkish	Deeper orange	N. decolor.	No change
(14) *Orange G (B):*				
0.01	Scarlet	Crimson	Brownish orange	No change
0.002	Pink	Brownish pink	Brown	No change
0.001	Pink	Brownish	Decolor.	Paler
(43) *Orange GT (SHH):*				
0.01	Red	Darker	Brownish orange	No change
0.002	Pink	Darker	Partly decolor.	No change
0.001	Pink	Darker	N. decolor.	No change
(84) *Resorcin Yellow (B):*				
0.01	Deep orange	Orange	Orange red	Slightly yellower
0.002	Orange	More yellow	Poor orange red	Slightly yellower
0.001	Orange	More yellow	N. decolor.	Slightly yellower
(85) *Orange I (SHH):*				
0.01	Deep violet	Blue violet	Deep violet red	Deep red
0.002	Violet	Violet	Red	Pink
0.001	Poor violet	Brownish	Slightly redder	Slightly pinker
(86) *Orange II (AD):*				
0.01	Crimson	Red violet	Orange red	No change
0.002	Pink	Violet	N. decolor.	No change
0.001	Pale pink	Brownish	Decolor.	N. decolor.

TABLE VIII.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

C. Yellows and Oranges

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(87) *Orange III (NA):*				
0.01	Violet red	Blue violet	No change	No change
0.002	Violet red	Blue violet	No change	No change
0.001	Violet red	Poor violet	No change	No change
(88) *Orange IV (B):*				
0.01	Dark red violet	Deep blue violet	Deeper	No change
0.002	Violet	Blue violet	Deeper	No change
0.001	Violet	Dirty bluish	Deeper	No change
(89) *Brilliant Yellow S (SHH):*				
0.01	Red violet	Magenta	No change	No change
0.002	Red violet	Magenta	No change	No change
0.001	Red violet	Magenta	No change	No change
(94) *Tartrazine (Ber.):*				
0.01	No change	No change	No change	No change
0.002	No change	No change	No change	No change
0.001	No change	No change	No change	No change
(95) *Metanil Yellow (B):*				
0.01	Red violet	Black violet	No change	No change
0.002	Red violet	Blue violet	No change	No change
0.001	Red violet	Dirty violet	No change	No change
(269) *Chrysamine R (SHH):*				
0.01	Red violet	Blue violet	Red orange	No change
0.002	Red violet	Blue violet	Red orange	No change
0.001	Red violet	Dirty violet	Red orange	No change
(425) *Auramine (Ber.):*				
0.01	Decolor.	N. decolor.	Decolor.	Paler
0.005	Decolor.	N. decolor.	Decolor.	Paler
0.002	Decolor.	N. decolor.	Decolor.	Paler
(510) *Uranine (AD):*				
0.01	More greenish	Deeper	Orange	Orange
0.005	More greenish	Deeper	Orange	Orange
0.002	More greenish	Deeper	Orange	Orange

TABLE VIII.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

C. Yellows and Oranges

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(667) *Quinoline Yellow (AD):*				
0.01	Darker	Brownish yellow	N. decolor.	No change
0.002	Darker	Brownish yellow	Decolor.	No change
0.001	Darker	Brownish	Decolor.	No change

D. Greens

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent. NaOH	Ammonia (0.96)
(398) *Naphthol Green B (B):*				
0.05	Yellow	Brownish yellow	No change	No change
0.001	Decolor.	Brownish yellow	No change	No change
0.005	Decolor.	Brownish	No change	No change
(427) *Malachite Green (Ber.):*				
0.01	Orange	Green to orange	Decolor.	Decolor.
0.002	Orange	N. decolor.	Decolor.	Decolor.
0.001	Orange	Decolor.	Decolor.	Decolor.
(428) *Brilliant Green (B):*				
0.01	Orange	Green to orange	Decolor.	Decolor.
0.002	Decolor.	N. decolor.	Decolor.	Decolor.
0.001	Decolor.	Decolor.	Decolor.	Decolor.
(435) *Light Green SF Yellowish (B)*				
0.01	Orange	Orange	Decolor.	Decolor.
0.002	N. decolor.	Orange	Decolor.	Decolor.
0.001	Decolor.	Orange	Decolor.	Decolor.

TABLE IX.—REACTIONS OF DYES IN AQUEOUS SOLUTION AND WITH CONCENTRATED SULPHURIC ACID

No.	HCl	NaOH	Color of dry dye	Dry color + sulphuric acid	
				Before dilution	After dilution
Blues and Violets:					
287	No change	Crimson	Violet	Blue	Violet
439	Green	More bluish	Blue	Pale yellow	Deeper yellow
440	Yellow green	No change	Greenish blue	Pale yellow nearly colorless	Dark yellow
451	Greenish	No change	Purple	Orange	Yellow green, then greenish blue
452	Green	No change	Purple	Orange yellow	Yellow green then greenish blue
464	Blue green to yellow	No change	Purple	Orange	Yellow green, then greenish blue
468	Green	No change	Violet	Orange	Yellow
476	No change	Pinker	Blue	Red	Blue (and blue ppt.)
480	No change	Redder	Blue violet	Red brown	Blue
585	Bluer	Redder	Red violet	Green	Red violet
601	Bluer	Redder	Violet blue	Blue violet	Violet blue
650	No change	No change	Blue violet	Green	Blue
692	No change	Bright green	Blue	Violet blue	Blue
Reds:					
53	No change	More orange	Red	Magenta	Rose pink
55	No change	Deeper, then decolorized	Red	Crimson	Red
56	Pinker	Yellower, decolorized with weaker solution	Red	Crimson	Rose pink
65	No change	Orange, dilute sol. decolor.	Violet	Blue	Magenta
71	No change	Orange	Red	Magenta	Pink
102	Yellow brown	No change	Red	Blue violet	Red orange
103	No change	More orange	Red violet	Blue violet	Red
106	No change	Red brown	Red	Red violet	Crimson
107	No change	Less pink	Red violet	Blue violet	Red
108	No change	Red brown	Brown	Blue violet	Crimson
160	No change	Violet brown	Red	Red violet	Brown ppt., then red ppt.
163	No change	Purple	Red	Bluish green	Red
169	No change	More violet	Violet red	Blue	Crimson
240	Blue	Orange	Red	Blue	Blue ppt.
277	Purple	More orange	Red	Blue	Blue ppt.
278	Violet	More orange	Red	Blue	Blue
448	Yellow	Decolorized	Purple	Orange	Yellow

TABLE IX.—REACTIONS OF DYES IN AQUEOUS SOLUTION AND WITH CONCENTRATED SULPHURIC ACID.—(*Continued*)

No.	HCl	NaOH	Color of dry dye	Dry color + sulphuric acid	
				Before dilution	After dilution
462	No change	Decolorized	Violet	Orange	Magenta
504	No change	No change	Violet	Yellow	Pink
512	Yellow fluor. destroyed	No change	Magenta	Yellow	Orange red
517	Yellow, then decolor.	No change	Violet	Orange	Pink
518	Decolorized	No change	Crimson	Orange	Colorless
520	Decolorized	No change	Violet	Orange	Faint pink
523	Decolorized	No change	Violet	Orange	Pale pink
584	More violet	No change	Violet red	Blue	Crimson
Yellows and Oranges:					
1	No change	No change	Yellow	Colorless	Yellow
3	Decolorized	No change	Red orange	Yellow	Pale yellow
4	Decolorized	No change	Orange	Yellow	Yellow
8	Pink orange	No change	Brownish	Orange	Red
13	No change	Redder	Orange red	Orange	Orange
14	No change	Pinker	Red	Crimson	Orange red
43	No change	No change	Orange red	Orange red	Orange
84	No change	Redder	Orange red	Orange	Orange
85	Darker	Brown red	Red brown	Blue violet	Magenta, then orange
86	No change	Reddish brown	Orange red	Red violet	Orange
87	Pinker	No change	Brown yellow	Blue violet	Red violet
88	Crimson	No change	Orange	Blue violet	Red violet
89	Darker	No change	Yellow	Red violet	Yellow brown
94	No change	No change	Orange	Yellow	Yellow
95	Magenta	No change	Brown	Blue violet	Red violet
269	Decolorized	Red orange	Orange	Blue violet	Pale yellow
425	No change	Decolorized	Yellow	Colorless	Yellow
510	Yellow fluor. destroyed	No change	Orange red	Yellow	Yellow
667	No change	No change	Yellow	Orange	Yellow
Greens:					
398	Paler	No change	Green	Orange	Yellow
427	Green, then orange	Decolorized	Blue green	Yellow	Red orange—green
428	Green, then orange	Decolorized	Green	Yellow	Red orange
435	Yellowish green	Decolorized	Green	Orange	Greenish blue

Separation of Mixtures.—Sometimes the color present is not a single dye, but a mixture of two or more is used to produce the shade desired, as green from a mixture of blue and yellow or red and yellow to produce orange.

The presence of such a mixture may usually be determined in dyeing on wool if the fractional dyeing as described on page 61 be employed. In the case of a mixture there will generally be a gradual change in the color of the wool from the first dyeings to the last. By combining the end fractions, stripping and re-dyeing, enough color can frequently be separated for identification.

A better method, however, is by extraction with immiscible solvents, being an elaboration of the method described on page 63. After separating the basic from the acid dyes by shaking out the former with amyl alcohol or ether from an alkaline solution, most of the acid colors can be extracted by amyl alcohol from the strongly acidified solution. If now the amyl alcohol be washed with successive portions of water, the dyes are removed at different stages, depending in general upon the degree to which they are sulphonated. Still others are removed from the amyl alcohol only by treatment with petroleum ether or by shaking out with dilute alkali.

Such a procedure, based very largely upon work done in the New York Food Inspection Laboratory,[1] is given below for the coal-tar dyes included in the table on page 59. In carrying out the extractions, it should be remembered that the degree of acidity and the consequent separation of the colors depends largely upon the comparative volumes used and the time of shaking, hence when these points are specified they should be closely followed. The solution to be tested should also be as free as possible from extraneous matter.

Procedure.—(*a*) *Separation of Basic Colors.*—Make a small portion of the color solution quite strongly alkaline with sodium hydroxide and shake with ether. Separate the ether and shake it with dilute acetic acid. If the ether is colored or yields a color to the acetic acid a basic color is probably present. If no color is noticed, make the acetic acid solution alkaline with ammonia and evaporate to dryness on the water-bath. A basic

[1] Mathewson: *Bur. of Chem., Bull.* **162,** p. 53.

dye which imparts no color to the ether or the acetic acid may usually be detected in this way.

If a basic color is found, add to the remainder of the original color solution one-fifth its volume of 10 per cent. sodium hydroxide and extract three times with half its volume of ether. *Avoid violent shaking and the consequent formation of troublesome emulsions.* Shake the combined ether extracts three times with half the volume of water. Preserve the first two extracts and reject the third. Finally, shake the ether twice with half its volume of dilute acetic acid (one part of acid, sp. gr. 1.04, to two of water). For the particular dyes that will be found at various points in this procedure consult the "Outline" page 85, remembering that to show the presence of the dye it may be necessary to evaporate the solution on the water-bath as described above.

(*b*) *Separation of Acid Colors.*—Add to 25 or 50 cc. of the original color solution one-half its volume of concentrated hydrochloric acid. If basic colors have been previously extracted the solution should be neutralized with hydrochloric acid before adding the excess of strong acid. Shake three times with amyl alcohol and combine the extracts, which should have a total volume not greater than that of the original solution used. Shake the amyl alcohol with successive portions of water of about one-half its volume until the last portions are perfectly neutral. Seven of these washings should be obtained and preserved for further examination. If color still remains in the amyl alcohol dilute it with two volumes of gasoline or petroleum ether and shake out twice with water as before. Separate further any colors obtained at this point as described in section (5) of the "Outline."

Finally shake the amyl alcohol-petroleum ether mixture twice with very dilute sodium hydroxide solution.

The original solution, from which basic dyes have been removed with ether and acid dyes with amyl alcohol, although perhaps perfectly colorless, may still contain a few colors, including the permitted dye Light Green S. F. Yellowish. Make the solution slightly alkaline with ammonia, acidify slightly with acetic acid and shake out twice with amyl alcohol. In case it is desired to separate still further the few dyes still re-

maining in the aqueous solution it may be extracted with small quantities of dichlorhydrin. This, however, is a rather expensive reagent and in the majority of cases will not be necessary. The separation is summarized in Group *C* of the "Outline."

Notes.—If any of the vegetable colors which are considered in the earlier part of this chapter are present, they will be extracted by the amyl alcohol from the strongly acid solution and appear at various points in the washing out with water. Logwood and saffron will be found mainly in fractions 1 and 2 and the petroleum ether fraction; cochineal will appear in Section 4 of the "Outline;" annatto, archil, Persian berries and turmeric will remain in the amyl alcohol even after the treatment with petroleum ether but are removed by the washing with dilute (2 per cent.) sodium hydroxide.

This method of separation depends upon the degree of sulphonation of the dyes and their consequent solubility. On washing the amyl alcohol solution with water, in general, the higher sulphonated dyes come out first, while the wash-water still contains much hydrochloric acid, the lower sulphonated ones later, and finally the unsulphonated acid colors as Erythrosine, Martius Yellow, etc. If a sufficient separation has not been thus effected, the fractions containing the chief amounts of each color may be united and put through the necessary part of the procedure again.

Outline

The following tabulation shows the points at which the various dyes will appear in the procedure. It must be remembered, however, that a given dye will usually appear in several washings and the table only indicates where the *maximum* amount will be found. The numbers of the dyes refer to the list on page 59.

A. Basic Dyes.—Extracted by ether from strongly alkaline solutions. (650 extracted only in small amount, perhaps with decomposition).

1. Readily removed from ether on washing with water. 448, 584, 585.
2. More or less slowly removed by water, quickly by acetic acid. 425, 427, 428, 451, 452, 504.

B. Acid Dyes.—Not extracted by ether. Extracted by amyl alcohol from the strongly acidified solution.

1. Removed in first washings of amyl alcohol, acidity high. (Mostly infractions 1 and 2): 8, 89, 108, 287, 692.
2. Removed at lower acidity, but usually above fourth-normal. (Mostly in fractions 2 and 3): 94, 106, 107.
3. Removed at rather low acidity. (Mostly in fractions 3, 4 and 5): 14, 53, 480.
4. Removed at very low acidity, but before washings are neutral. (Mostly in fractions 4, 5 and 6): Combine the most deeply colored fractions, add one-half volume of HCl and shake out with amyl acetate.
   (*a*) Readily extracted: 1, 4, 160, 163, 169, (278).
   (*b*) Not readily extracted: 55, 56, 65, 84, 103.
5. Removed by water from the practically neutral solvent, most readily after the addition of petroleum ether. (Fractions 6, 7 and the petroleum ether fractions.) Combine the most deeply colored fractions, add ⅕ volume of HCl (1.20) and shake out with an equal volume of ether.
   (*a*) Almost no color extracted. Add 5 per cent. of NaCl and shake with amyl alcohol. Separate the amyl alcohol and shake it with 5 per cent. Na_2CO_3 solution.
      (*x*) Almost completely extracted: 85.
      (*y*) Not readily extracted: 87, 88, 95, 667.
   (*b*) Almost all extracted: 13, 43, 71, 86, (87), 102, 277, (278).
6. Removed by dilute sodium hydroxide. 3, 269, 510, 512, 517, 518, 520, 523.

C. Not extracted by ether. Not extracted from the strongly acidified solution by amyl alcohol. (Decomposed: 398.)

Add NH_4OH until slightly alkaline, then acetic acid until slightly acid and shake out with amyl alcohol.

(*a*) Readily extracted: 464, 468 (650).

(*b*) Not readily extracted. Shake with small quantities of dichlorhydrin.

1. Readily extracted: 240, 435, 439, 440.
2. Not readily extracted: 462.

Systematic Examination of a Food Product for Color.—In the examination of a product suspected to have added color present, it is usually best, both on account of the comparative ease with which the test can be carried out on an impure solution or suspension of the material, and because of the greater frequency with which the acid coal-tar dyes are employed, to make first the dyeing test on wool as described on page 60. Especial care should be taken to note the possible presence of a

mixture of coal-tar colors. If such should be the case, it will generally be found advisable to separate the colors by a systematic procedure as given on page 83. The possibility of a basic color being present should not be overlooked.

If a coal-tar color is not found or if the color of the material cannot all be accounted for by the presence of such a color, vegetable color should be sought. This may be conveniently separated from the material by extraction with amyl alcohol and the color identified by the tables and special tests on pages 53 to 58. It will usually be necessary to test for only a limited number of the vegetable colors, cochineal, for example, being hardly to be expected in a lemon extract or turmeric in currant jelly.

If the presence of artificial color is still suspected and none has been found up to this point, there still remain the pigments or mineral colors and the examination of some classes of food materials for added color would not be complete unless search had been made for this group of colors, usually by testing the ash for aluminum, tin, etc.

In the case of coal-tar colors, it should be borne in mind that the tables given in this chapter include only a selected list of colors so that one may be found which does not coincide in all its reactions with any of those tabulated. The seven permitted dyes are naturally the ones which will be found of most frequent occurrence.

Chemical Preservatives

General.—From ancient times certain methods and materials have been used in the treatment of foods in order to prevent fermentation and decay, and from an economic standpoint such preservation is necessary in order to conserve for future use such food as cannot be consumed at the time or place where produced in the greatest abundance. So far as the consumer is concerned, however, an important feature in regard to the use of these older methods, such as smoking, salting or pickling, is that the use of the preservative is at once evident to the senses, being recognized by the taste or odor. Such materials as sodium benzoate, salicylic acid and the like, on the other hand,

when present in food in amounts sufficient for preservation, being tasteless and odorless, are not thus evident to the consumer, who must rely upon the label or upon the result of chemical analysis. The detection of preservatives in food is hence of much importance.

The most common of the "chemical" preservatives are *formaldehyde*, *salicylic acid*, *sodium benzoate*, *sulphurous acid* and *sulphites*, *borax* and *boric acid*, and *fluorides*. Saccharin, although commonly employed as a sweetening agent, has a distinct inhibitory effect on bacterial action and may properly be included. The various individuals are added most frequently to particular foods, as formaldehyde and borax to milk, salicylic acid to fruit juices, sodium benzoate to ketchup, jams and sauces, sulphites to dried fruits, gelatin and chopped meat, and fluorides to beer. Some of them, as borax, salicylic and benzoic acids, and fluorides, occur naturally in notable amounts in certain food products, so that their mere presence is not always enough to show that preservative has been *added*.

For a full discussion of the harmfulness of food preservatives or their desirability in foods, reference must be made to larger works and to official publications. Some of the principal arguments urged by those who would allow their use are: That they are used in such minute amounts as to be in no way harmful; that any possible danger from their use would be far less than from the use of foods partially spoiled on account of not having preservatives added; that foods in which these same chemical substances occur naturally are used with no injury resulting.

Those opposed to the use of preservatives urge: That their use is unnecessary, since if some manufacturers can prepare food without preservatives, all can; that their use is a constant temptation for unscrupulous manufacturers to put on the market unsuitable food, already partly decayed, but the fermentation arrested by the help of preservatives; that a preservative capable of arresting fermentation or bacterial action must have some degree of unwholesome effect on the process of digestion, since this is largely due to the action of enzymes and bacteria.

Formaldehyde

Formaldehyde is a gaseous product of the partial oxidation of methyl alcohol, and is used as a food preservative as a dilute solution (2 to 30 per cent.) in water. In the case of some food products, as milk, tests may be applied directly; with others, where interfering substances may be present, a portion should be acidified with phosphoric acid, distilled, and the first portion of the distillate tested by one of the methods given below.

1. **Hehner's Sulphuric Acid Test.**[1]—To 5 cc. of milk in a test-tube add an equal volume of the liquid to be tested, and pour carefully 5 cc. of concentrated commercial sulphuric acid down the side of the tube so that it shall not mix with the diluted milk. In the presence of formaldehyde a violet ring is formed at the junction of the two liquids. The delicacy of the test is about one part in 200,000.

Note.—Pure sulphuric acid does not give the test, which is dependent upon the presence of a small amount of oxidizing agent, as the ferric salts present in the commercial acid. If only pure acid is available, a little ferric chloride should be added.

The reaction is a general one for proteids, depending upon the presence of the tryptophane group in the protein molecule.[2]

2. **Hydrochloric Acid Test.**[3]—Mix 5 cc. of the liquid to be tested with 5 cc. of pure milk in a small casserole, add 10 cc. of concentrated hydrochloric acid (containing 0.2 gram of ferric chloride per liter) and heat slowly nearly to boiling, occasionally giving the casserole a rotary movement to assist in dissolving the curd. Keep just below the boiling point for 1 minute, add 50–75 cc. of water and note the color carefully. In the presence of formaldehyde a violet color is produced, showing best at the moment of dilution.

Note.—The reaction which occurs in this test is undoubtedly the same as in the preceding. Owing, however, to the absence of the charring effect due to the sulphuric acid, the test is more delicate, showing one part of formaldehyde in 250,000.

[1]Hehner: *Analyst*, **1896**, 95.

[2]Rosenheim: *Analyst*, **1907**, 106.

[3]Leach: *Ann. Rept. Mass. State Board of Health*, **1897**, 558; Sherman: *School of Mines Quarterly*, **1905**, 408.

3. Gallic Acid Test.[1]—This test has been found by Sherman[2] to be much more delicate than either of the preceding tests. Twenty-five to 50 cc. of the material should be acidulated with phosphoric acid and distilled. To the first 5 cc. of the distillate add 0.2 to 0.3 cc. of a saturated solution of gallic acid in pure ethyl alcohol and pour it cautiously down the side of an inclined test-tube containing 3–5 cc. of pure concentrated sulphuric acid. If formaldehyde is present a green zone is formed at the junction of the two layers, gradually changing to a pure blue ring.

The delicacy of the test is about one part of formaldehyde in 500,000.

Benzoic Acid and Sodium Benzoate

Benzoic acid, in the form of its sodium salt, is probably the preservative most widely used in different varieties of food at present. This is partly due to its suitability for a wide range of products and partly to the fact that it is permitted in food products under the Federal Pure Food Law, provided that not more than 0.1 per cent. is used and that the presence and amount are declared on the label.

Detection.—If the material is a liquid, 25 or 50 cc. may be acidified with 5 cc. of dilute sulphuric acid and shaken with 25 cc. of ether in a separatory funnel. In shaking, mix the liquids thoroughly by tipping the funnel back and forth ten or twelve times, but avoid *violent* shaking which tends to form emulsions, especially with saccharine liquids. If an emulsion forms, it can often be separated by drawing off the clear aqueous layer as far as possible and then giving the funnel a quick, vigorous shake. If this does not suffice, add 10 cc. of gasoline or petroleum ether and shake again, or centrifuge the mixture in the machine described on page 29. The ether solution should be as clean and as free from the aqueous layer as possible.

If the original material is very thick, or solid, a corresponding quantity should be diluted or macerated in a mortar with water and strained, to obtain a solution which is acidified and extracted as above.

[1]Barbier and Jandrier: *Ann. Chim. Anal.*, **1896**, 325; Mulliken and Scudder: *Am. Chem. J.*, **1900**, 444.

[2]*J. Am. Chem. Soc.*, **1905**, 1499.

The ether layer is separated and evaporated in a casserole or porcelain dish at room temperature or at a *low* heat. *Do not bring it near a flame.* If the original material contained much fat, which would be taken up by the ether, the preservative is best obtained by shaking the ether with dilute ammonia, the ammonia removed by evaporation and the residue tested.

If considerable benzoic acid is present, it may be recognized in the residue from the ether as crystalline scales having a characteristic odor when heated. The residue should be tested as follows:

Mohler's Method.[1]—Add to the residue 2–3 cc. of concentrated sulphuric acid and heat until the acid fumes strongly; the organic matter is charred and benzoic acid changed to sulphobenzoic acid. Add ammonium nitrate in small portions to the acid while still fuming; the organic matter is oxidized and *m*-dinitrobenzoic acid formed. Let the solution cool, add a little water and ammonia in considerable excess and transfer a portion of the solution to a test-tube. Pour a few cc. of *freshly prepared* colorless ammonium sulphide down the side of the tube so as not to mix with the ammoniacal solution. A red ring, due to the formation of ammonium *m*-diamidobenzoate, is formed at the junction of the two liquids if benzoic acid is present. To the remainder of the solution in the casserole add one or two drops of the ammonium sulphide and the red color will form in clouds on the surface.

Note.—Cinnamic acid, which may be present in foods as an oxidation product of the cinnamic aldehyde of cinnamon oil in the spice used, acts similarly to benzoic acid in this test.[2] It may, however, be detected by heating the ether residue to boiling with dilute chromic acid mixture, when the cinnamic acid or cinnamic aldehyde will be oxidized to benzaldehyde, recognized by its characteristic almond odor,[3] or the modified method below may be employed. (See also page 90.)

Mohler's Method Modified by von der Heide and kakob.[4]—Take up the ether residue in 1 to 3 cc. of third-normal sodium

[1] *Bull. Soc. Chim.*, **1890**, 414
[2] Scoville: *Am. J. Pharm.*, **79**, 549.
[3] Bigelow and Dunbar: *Bur. of Chem., Bull.* **122**, p. 77.
[4] *Z. Nahr. Genussm.*, **1910**, 137.

hydroxide and evaporate to dryness. To the residue add 5 to 10 drops of concentrated sulphuric acid and transfer as thoroughly as possible to a small test-tube. Add a crystal of potassium nitrate and heat for 10 minutes in a glycerin or oil bath at 120° to 130°C., or for 20 minutes immersed in boiling water. In no case should the temperature exceed 130°C. After cooling, add 1 cc. of water and make decidedly ammoniacal; boil the solution to break up any ammonium nitrite which may have formed. Cool and add a few drops of fresh colorless ammonium sulphide, without allowing the layers to mix. A red-brown ring indicates benzoic acid. On mixing, the color diffuses through the whole liquid; on heating it finally changes to greenish yellow, owing to the decomposition of the amido acid.

Notes.—The reactions which take place are the same as in the original method, but the modified method is more delicate and reliable, since the conditions are more definitely controlled. Salicylic and cinnamic acids give a similar reaction except that the amido compounds formed do not decompose on heating, so that the change to the greenish-yellow color does not take place with these. It is to be remembered that the benzoic acid residue should be reasonably pure, since any large amount of organic matter interferes with the test. Phenolphthalein also interferes, but is not likely to be present in a food product.

Quantitative Determination.—The simplest method of determining small amounts of benzoic acid or benzoates in food is by extracting with a suitable immiscible solvent and titrating the extracted benzoic acid. Chloroform, while not so good a solvent for benzoic acid as ether, is preferable because it is not inflammable and dissolves only traces of mineral acids, tannin, salts, and other interfering substances. Further, it is heavier than water and can conveniently be drawn off from the bottom of the separatory funnel. By taking advantage of the "salting out" principle by using a saturated solution of sodium chloride, the benzoic acid is rendered much less soluble in the aqueous liquid and the extraction may be made quantitative.

Certain other organic acids, principally acetic, may be extracted in slight amount by the chloroform, but if the extract is evaporated and dried the error from this cause is negligible.

A. **Preparation of the Solution.**[1]—*General Method.*—Grind in a sausage machine or food chopper if solid or semisolid and mix thoroughly. Transfer about 150 grams to a 500-cc. flask, add enough fine table salt to saturate the water in the sample, make alkaline with sodium hydroxide or milk of lime and make up to the mark with saturated salt solution. Allow to stand at least 2 hours with frequent shaking and filter. If the sample contains large amounts of matter precipitable by salt solution, it is best to proceed as described under (4) of the "Special Methods." If alcohol is present, follow the method described under (3) of the "Special Methods." If much fat is present, it is well to make the filtrate alkaline and extract with ether before proceeding with the extraction of the benzoic acid.

Special Methods.—1. *Ketchup.*—To 150 grams of sample, add 15 grams of fine table salt. Transfer the mixture to a 500-cc. graduated flask, using about 150 cc. of saturated salt solution for rinsing. Make slightly alkaline to litmus paper with strong sodium hydroxide and make up to 500 cc. with saturated salt solution. Allow to stand at least 2 hours with frequent shaking and filter through a large folded filter. If filtration is difficult, the mixture may be centrifuged before filtering.

2. *Jellies, Jams, Preserves and Marmalades.*—Dissolve 150 grams of the sample in about 300 cc. of saturated salt solution. Add 15 grams of table salt. Make alkaline to litmus paper with milk of lime. Transfer to a 500-cc. graduated flask and fill to the mark with saturated salt solution. Allow to stand at least 2 hours with frequent shaking, centrifuge if necessary, and filter through a large folded filter.

3. *Cider and Similar Products Containing Alcohol.*—Make 250 cc. of the sample alkaline to litmus paper with sodium hydroxide and evaporate on the steam bath to about 100 cc. Transfer to a 250-cc. flask, add 30 grams of table salt and shake until dissolved. Dilute to the mark with saturated salt solution, allow to stand at least 2 hours with frequent shaking, and filter through a folded filter.

4. *Salt or Dried Fish.*—Transfer 50 grams of the ground sample to a 500-cc. flask with water. Make slightly alkaline

[1]Dunbar: *Bur. of Chem., Bull.* **132,** p. 138.

to litmus paper with strong sodium hydroxide and dilute to the mark with water. Allow to stand at least 2 hours with frequent shaking and filter through a folded filter. Measure 300 cc. of the filtrate into a second 500-cc. flask, add 90 grams of table salt, shake until dissolved, and fill to the mark with saturated salt solution. Mix thoroughly and filter off the precipitated proteins on a folded filter.

B. **Extraction and Titration.**—Measure a convenient portion (100 to 200 cc.) of the filtrate obtained by one of the above methods, into a separatory funnel. Neutralize to litmus paper with hydrochloric acid (1:3) and add 5 cc. in excess. With salt fish, protein matter usually precipitates on acidifying, but this does not interfere with the extraction. Extract carefully with chloroform, using for a 200-cc. portion of the filtrate successive portions of 70, 50, 40 and 30 cc., and proportional amounts for smaller aliquots. To avoid emulsions, shake each time cautiously; vigorous shaking is unnecessary. The chloroform layer usually separates readily (at the bottom) after standing a few minutes. If any emulsion forms, it can be broken by stirring the chloroform layer with a glass rod. If this does not succeed, draw off the emulsified portion into a second funnel and give it one or two sharp shakes. If this also fails, centrifuge the emulsion several moments. As this is a progressive extraction, great care must be taken to draw off as much of the clear chloroform layer as possible each time, but on no account draw off any of the emulsion at any time. If not contaminated with the emulsion, it is not necessary to wash the chloroform extract.

Transfer the combined chloroform extracts to a shallow porcelain or glass dish, rinsing several times with small portions of chloroform, and evaporate to dryness at room temperature in a current of dry air. A blast of air which has passed through a bottle containing calcium chloride, or an electric fan, is convenient. Dry the residue over night (or until no odor of acetic acid can be detected, in the case of ketchup) in a sulphuric acid desiccator. Dissolve the residue of benzoic acid in 30 to 50 cc. of neutral alcohol, add about one-fourth the volume of water, two drops of phenolphthalein solution, and titrate with twentieth-normal sodium hydroxide.

Salicylic Acid

Detection.—The solution of the food material for the qualitative test for salicylic acid may be prepared in the same manner as directed for the detection of benzoic acid, page 93, except that in no case should the amount of material used exceed 50 grams. Since the two preservatives occur in the same class of products, it is often advisable simply to divide the ether extract into two portions and test one for salicylic acid and the other for benzoic acid. In this case, since the test for benzoic acid is more difficult than that for salicylic, the larger portion should be tested for the former preservative. The portion of the ether extract which is to be tested for salicylic acid should be quite free from the aqueous layer, and since the ferric test for salicylic acid is less delicate in the presence of mineral acids, it should be freed from traces of sulphuric acid by washing it twice with one-tenth its volume of water. Transfer the ether to a porcelain dish, evaporate it spontaneously or at low temperature, and test the residue by the following tests:

1. **Ferric Chloride Test.**—To the residue add 2 drops of dilute (1 per cent.) ferric chloride solution, or better, 3–4 drops of ferric alum solution[1] and rub it around with a blunt glass rod. A violet color is produced in the presence of salicylic acid. The test is delicate with ordinary care to 0.50 milligram of salicylic acid but is not conclusive of the presence of the preservative, since the color is not characteristic but is given by several other organic substances. In case of a positive result, therefore, the conclusion may be confirmed by the following test which is recommended by Sherman[2] as highly satisfactory:

2. **Jorissen Test.**[3]—To the solution to be tested add 4 or 5 drops of a 10 per cent. solution of potassium (or sodium) nitrite, 4 or 5 drops of acetic acid, 1 drop of 10 per cent. solution of copper sulphate, and heat to boiling. In the presence of sali-

[1] Dissolve 2 grams of ferric alum in 100 cc. of water, heat to boiling and boil for a moment or two, allow to settle and filter. The reagent is preferable to ferric chloride in that a slight excess does not interfere with the delicacy of the test.

[2] Organic Analysis, 2d Ed., p. 381.

[3] Jorissen: *Bull. l'Acad. Sci. Belg.*, **3**, 259; Sherman: *J. Ind. Eng. Chem.*, **1911**, 24; Sherman and Gross: *Ibid.*, **1912**, 492.

cylic acid the solution takes on a reddish tinge, and with more than a very minute amount becomes blood-red. Phenol gives the same reaction but benzoic acid does not.

By diminishing the amount of copper used and by heating for a longer time, the test can be made more delicate but this is hardly necessary for ordinary food work.

Notes.—A small amount of salicylic acid occurs naturally in some fruits, hence not more than 50 grams of food should be used if it is desired to show added salicylic acid.

As Sherman[1] carefully points out, it is not safe in testing foods to assume that a constituent volatile with steam, soluble in ether, capable of sublimation and crystallization, and giving a violet reaction with ferric salts, is necessarily salicylic acid. Highly roasted malt and baked food products which may have been partly caramelized have been reported to contain a substance ("maltol") which corresponds to salicylic acid in all these properties.[2] The Jorissen test, however, serves to distinguish salicylic acid from this and practically all the other substances which give a violet color with ferric chloride.

Quantitative Determination.—Salicylic acid is ordinarily added to foods in so much smaller quantity than benzoic acid that it is not feasible to determine it by titration. Recourse is therefore had to its colorimetric estimation, the method of extraction and estimation being very similar to the qualitative examination. The extracted salicylic acid must be carefully freed from accompanying substances, chiefly tannins, which give similar reactions and hence would interfere. The following method is the one adopted by the Association of Official Agricultural Chemists.[3]

Procedure.—Macerate 200–300 grams of the sample in a mortar or porcelain dish with water containing a trace of alkali, transfer the mass gradually to a 500-cc. graduated flask, make up to the mark with water and shake at intervals until all soluble matter has dissolved. Strain through cloth, or centrifuge.

[1] *Loc. cit.*

[2] Brand: *Z. ges. Brauw.*, **15**, 303; Backe: *Ann. des falsifications*, **Nov., 1909**; Sherman: *J. Ind. Eng. Chem.*, **1911**, 24.

[3] *Bur. of Chem., Bull.* **107** (Revised), p. 179.

Place 100 cc. of the solution in a separatory funnel, add 3 cc. of dilute (1 : 3) sulphuric acid and extract with 50 cc. of ethyl ether. Use the precautions in shaking or in breaking emulsions that are given on page 90. Separate the aqueous layer as completely as possible and transfer the clear ether layer to a second separatory funnel, taking care that no emulsion or none of the aqueous solution remains with the ether. Return the aqueous portion to the funnel and extract three times more in the same way, using 50 cc. of ether each time.

In case of special difficulty in breaking up the emulsion in any of the extractions, a small amount of ether may be allowed to remain with the aqueous portion rather than the reverse, as it is removed in successive extractions. Wash the combined ether extracts twice by shaking in a separatory funnel with one-tenth their volume of water (using, however, not less than 20 cc. of water at each washing). Care must be taken at each washing to separate the aqueous portion completely from the ether, but none of the ether should be allowed to run into the wash water.

Distil slowly the greater part of the ether, transfer the remainder to a porcelain dish and allow it to evaporate spontaneously. Thoroughly dry in a vacuum desiccator over sulphuric acid and extract the dry residue with ten portions of 10 or 15 cc. each of carbon bisulphide or low-boiling petroleum ether (gasoline), rubbing the contents of the dish with a glass rod or other suitable instrument and transferring the successive portions of solvent to a second porcelain dish. The extracted residue should finally be tested with a drop of ferric-alum solution, and if any reaction for salicylic acid be given, it should be taken up with water, re-extracted with ether, and the operation repeated. The petroleum ether extract is finally allowed to evaporate spontaneously.

Dissolve the residue in a small amount of hot water and dilute to a definite volume. Dilute aliquots of the solution, add 2 drops of ferric chloride (0.5 per cent.) or 3–4 drops of ferric alum and compare the color with that given by a standard solution of salicylic acid (1 mg. in 50 cc.), using Nessler tubes or a colorimeter. In either case, and especially with ferric chloride, an excess of reagent should be avoided.

Notes.—If the nature of the substance is such that extraction with ether is objectionable, as would be the case if a large amount of fat were present, the sample may be acidified with phosphoric acid and the salicylic acid distilled from an oil-bath at a temperature of 120°–130°C.[1]

In examining a substance whose ether extract does not, in the absence of salicylic acid, give a color or precipitate with the ferric solution, the drying of the residue and its extraction with petroleum ether may be omitted. The residue may be transferred by means of warm water directly from the flask from which the ether was distilled to the graduated flask, in which it is made up to a definite volume.

In the case of ketchup and similar foods, which give to the petroleum ether a color which interferes with the ferric test, good results may often be obtained by making the solution alkaline with lime water and filtering before extracting with ether. In this way tannin and other objectionable substances are removed.[2]

Boric Acid and Borax

Qualitative Tests.—The well-known test with turmeric paper is the most convenient as well as the most delicate for the detection of boric acid and borates in food products. It may be carried out in the following manner:

Make about 25 grams of the sample distinctly alkaline with lime water or sodium hydroxide and evaporate to dryness in a porcelain or platinum dish. Ignite the residue until charred, boil with about 15 cc. of water, add hydrochloric acid drop by drop until acid to litmus paper, then add 10 drops in excess. Filter and evaporate to dryness on the water-bath with a strip of turmeric paper immersed partly in the liquid. In the presence of borax or boric acid, the dry paper will be colored cherry red, which is changed by a drop of ammonia to bluish green.

Note.—By carrying out the evaporation and drying at room temperature in a desiccator, the test can be made considerably

[1] *Bur. of Chem., Bull.* **107,** p. 179.

[2] Dubois: *J. Am. Chem. Soc.,* **1906,** 1916.

more delicate[1] but this increased delicacy is of little value if it is desired to test only for boric acid *added as a preservative.* Boric acid in small quantities is widely distributed in nature, apples and other fruits and vegetables showing distinct traces of it. It occurs also in common salt, some kinds of which contain considerable amounts. In doubtful cases, therefore, it is best to determine the boric acid quantitatively.

Quantitative Determination.[2]—To 50 or 100 grams of the sample, add enough sodium hydroxide (1 or 2 grams) to render it decidedly alkaline and evaporate to dryness in a platinum dish. Cautiously, but thoroughly, char the residue and boil with 20 cc. of water, adding hydrochloric acid drop by drop until all but the carbon is dissolved. Wash into a 100-cc. graduated flask, keeping the volume below 50–60 cc. Add 0.5 gram of dry calcium chloride, a few drops of phenolphthalein, then a 10 per cent. solution of sodium hydroxide until a slight permanent pink color is produced, and finally add 25 cc. of lime water. All the phosphoric acid has thus been precipitated as calcium phosphate. Make up to 100 cc., shake and filter through a dry filter. To 50 cc. of the filtrate, add normal sulphuric acid until the pink color just disappears, then add a few drops of methyl orange and continue the addition of sulphuric acid until the yellow is just turned to pink. At this point all the acids are present as salts neutral to phenolphthalein except boric acid and carbonic acid. Boil the solution 2 or 3 minutes to expel carbon dioxide. Tenth-normal sodium hydroxide is next added until the liquid just assumes the neutral yellow color, avoiding excess. Cool the solution, add a little more phenolphthalein and as much glycerin as the volume of the solution. Titrate with tenth-normal sodium hydroxide to a permanent pink color. Each cc. of sodium hydroxide used in the final titration is equal to 0.0062 gram of boric acid.

Notes.—Although alkali is added before ignition there is a possibility of some boric acid being volatilized through imperfect mixing of the alkali with the food. Hence it is best to ignite no more than is necessary to char the material thoroughly in order that it may be exhausted with water.

[1] Low: *J. Am. Chem. Soc.*, **1906**, 805.

[2] Thomson: *Glasgow City Anal. Soc. Rept.*, **1895**, p. 3.

Boric acid cannot be titrated by means of the ordinary indicators on account of its feeble ionization. Hence the addition of the glycerin, which forms with the acid a complex which is much more highly ionized than the boric acid itself. Two or 3 grams of mannitol can be used instead of the glycerin if preferred.

Sulphurous Acid and Sulphites

Sulphurous acid is generally employed in the preservation of food products in the form of acid sulphites, especially of sodium or calcium, or it may be present in the form of the sulphur dioxide gas itself. This is the case especially with such products as wines or molasses where the acid is probably present partly in combination with aldehydes or sugars.

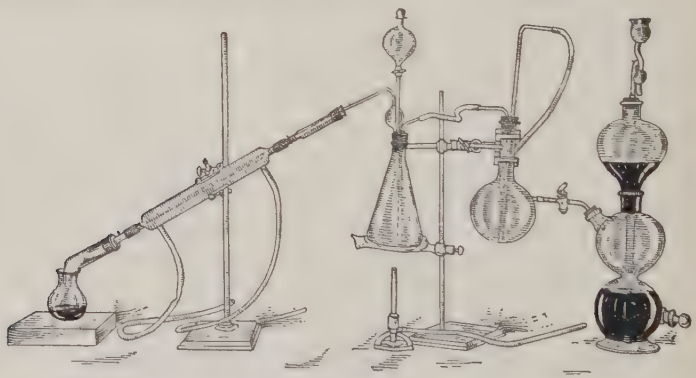


Fig. 37.—Apparatus for determining sulphurous acid.

Detection and Determination.—Weigh 25 to 100 grams of the sample into a 500-cc. flask arranged as shown in Fig. 37. If a solid, add recently boiled water. Through the separatory funnel add 5–10 cc. of a 20 per cent. solution of a phosphoric acid and distil by steam in a current of carbon dioxide, collecting the distillate in 25 cc. of saturated bromine water. Distil about 150 cc. adding a little more bromine from time to time if necessary. The end of the condenser or the adapter should dip beneath the surface of the bromine water. After the air has been expelled and the apparatus filled with steam, the current of carbon dioxide may be shut off. A small flame should be kept under the

flask containing the sample in order to avoid undue condensation of the steam, and enough heat applied to the round-bottomed flask to keep the water boiling briskly. The delivery tube from the flask should extend some distance into the condenser and rubber corks should be avoided at all points at which the bromine vapor may come into contact with them.

After the distillation is complete, boil off the excess of bromine, dilute the solution to 250 cc., add 5 cc. of hydrochloric acid (sp. gr. = 1.12), heat to boiling and add very slowly, with constant stirring, a hot, 10 per cent. solution of barium chloride until in slight excess. Allow to stand for an hour, filter, wash with hot water, ignite and weigh as barium sulphate.

A "blank" determination should be run with none of the food material in the flask, and any barium sulphate obtained subtracted from the main amount.

Notes.—This method determines the *total* sulphurous acid, both free and combined. If it is desired to determine the free acid only, the same procedure may be used, except that no phosphoric acid is added.

In exceptional cases, as in some canned foods, sulphides may possibly be present as well as sulphites. The hydrogen sulphide evolved upon the addition of phosphoric acid can readily be removed by interposing between the flask and the condenser a small flask containing a 1 per cent. solution of copper sulphate.[1]

The method may be simplified for use as a qualitative test by omitting the current of carbon dioxide, adding 10–15 cc. of phosphoric acid instead of the smaller quantity, and dropping into the distilling flask about a gram (not more) of sodium bicarbonate immediately before attaching to the condenser.

Fluorides

The fluorides of sodium and ammonium have been used to some extent as food preservatives, especially in beer and malt extracts. The most satisfactory method for their detection is by the well-known etching of glass.

[1] Winton and Bailey: *J. Am. Chem. Soc.*, **1907**, 1499.

Method.—If the sample is a liquid, use 150 cc. of it. Add 10 cc. of potassium sulphate (33 grams per liter), heat the solution to boiling and while boiling add drop by drop 10 cc. of a 10 per cent. solution of barium acetate. Boil for a minute, allow to settle and decant off the clear supernatant liquid. Wash the precipitate once with water by the aid of a centrifuge, thus avoiding the use of filter paper, which sometimes contains traces of fluorides, transfer to a platinum crucible, dry and ignite gently to destroy organic matter.

If the substance is a solid, mix it with a small quantity of sodium carbonate and burn to an ash, then proceed with the addition of sulphuric acid, as directed below.

Meanwhile a small glass plate, clear and free from scratches, is thoroughly cleaned and coated on one side with a mixture of equal parts paraffin and carnauba wax. This can readily be done by pouring a little of the melted wax on the warmed plate. If the excess is drained off and the glass held level, a thin uniform wax coating will result. While the coating is still warm, make a characteristic mark, such as a small cross (+), in the wax with a pointed instrument, taking care that the glass is laid bare but not scratched. The precaution should be taken to have the cross of approximately the same size in the different tests; for example, with arms about 4 mm. long and 1 mm. wide. On the uncoated side of the plate locate the cross by marks placed at the ends of the arms with a diamond or file.

To the precipitate in the crucible add 2 or 3 cc. of concentrated sulphuric acid, hold the crucible in the tongs, and heat its upper edge cautiously and quickly in a small flame. Press the glass plate quickly down on the warm crucible, having the cross nearly in the center, and hold it a moment to seal the glass securely to the crucible. The crucible should be embedded in the wax so firmly that it can be lifted by the plate. Support the crucible in a piece of heavy asbestos board in which a hole has been cut so that the crucible fits closely, put two or three drops of water on the glass plate and press down on it a condenser, the lower end of which is closed by a piece of thin sheet rubber such as is employed by dentists. Heat the crucible for an hour by a small flame about 9 mm. long placed 6 mm. below

the bottom of the crucible. At the end of this time remove the plate, scrape off the wax and clean the glass on both sides with "bon-ami" or other polishing material which will not scratch the glass. Examine it by reflected light for an etching. A test should not be considered positive unless the cross can be seen when viewed from either side of the glass.

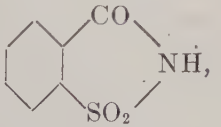
Notes.—It is hardly necessary to speak of the need for using the purest reagents obtainable and testing them carefully by blank tests.

The condenser may be made from a piece of wide glass tubing or a "carbon funnel" arranged so that a constant current of cold water may be kept flowing through it and with a piece of thin sheet rubber (dental dam), stretched tightly over the bottom. The diameter of the tube should be somewhat greater than that of the platinum crucible.

The delicacy of the procedure above described, as tested on aqueous solutions of potassium fluoride, is rather surprising. A perfectly distinct etching is readily obtained from 150 cc. of solution containing 1 : 10,000,000 of fluorine, and by careful working it is possible to get a recognizable test from 1 : 50,000,000. By a recognizable test is meant one which is visible from either side of the glass and does not have to be brought out by breathing on the glass. Care should be taken to clean the glass with "bon-ami" before examining it, since sulphuric acid alone will give a "stain" which can be brought out by breathing on it, and might easily be mistaken for an etching. The scouring will always remove this, however.

It is possible, by controlling more closely the temperature during the etching, to make the method approximately quantitative and this should be done in doubtful cases on account of the wide distribution of traces of fluorine in natural products.[1]

Saccharin

Saccharin, or benzoyl sulphimide, 

[1] Woodman and Talbot: *J. Am. Chem. Soc.*, **1906,** 1438; **1907,** 1362.

although better known as the first of the artificial sweeteners to be used commercially, possesses a certain degree of antiseptic power and hence may be included among the preservatives. The Referee Board of Consulting Scientific Experts having found that the continued use of saccharin for a long time, in quantities over 0.3 gram per day, is liable to impair digestion, its use in foods has been prohibited under the Federal Food Laws.[1]

The simplest method of testing for saccharin is to extract the material with ether and note whether the residue left after evaporating the ether has an extremely sweet taste. A more delicate test is to convert the saccharin to sodium salicylate and test for salicylic acid. This latter test obviously can be applied only in the absence of salicylic acid. There is occasionally present in some food materials a substance, the so-called "false saccharin," which also responds to this test and must be removed.

Preliminary Test.—Extract with ether as described under salicylic acid. Evaporate the ether spontaneously and taste of the residue. The residue will taste sweet in the presence of saccharin to the amount of 20 milligrams per liter.

Confirmatory Test.—Acidify 50 cc. of a liquid food or the water solution of 50 grams of a solid, prepared as directed under salicylic acid, and extract with ether. Test the ether extract for salicylic acid as described on page 95. If petroleum ether was used to purify the residue, return the petroleum ether to the dish containing the residue, evaporate, dilute to about 10 cc. and add 2 cc. of sulphuric acid (1 : 3). Bring the solution to the boiling point and add a 5 per cent. solution of potassium permanganate, drop by drop, to slight excess. Partly cool the solution, dissolve it in a piece of sodium hydroxide and filter into a small silver dish (a porcelain crucible can be used). Evaporate to dryness and heat for 20 minutes at 210° to 215°C., using an oil- or air-bath. Dissolve the residue in water, acidify and extract with ether, evaporate the ether, and test the residue with 2 drops of a 2 per cent. solution of ferric alum.

Note.—By this method all the so-called false saccharin and the salicylic acid naturally present (also added salicylic acid when

[1] U. S. Dept. Agr., Food Inspection Decision 135.

not present in too large amount) are destroyed, while 5 milligrams of saccharin per liter can be detected with certainty.

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Wiley.—Influence of Food Preservatives and Artificial Colors on Digestion and Health. U. S. Dept. of Agr., Bur. of Chem., Bull. 84.

For a selected list of recent journal references on the detection of preservatives consult Sherman's Organic Analysis, 2d Edition.

CHAPTER IV

MILK AND CREAM

Milk

Milk is a food material of somewhat complex and variable composition but can be described as essentially an aqueous solution of milk sugar, mineral salts and soluble albumin containing suspended globules of fat and partially dissolved casein.

Of these constituents, *milk sugar* or lactose is more fully discussed in the chapter on Carbohydrate Foods, page 230, and the *fat* is treated separately under Butter, page 200. The *casein*, which comprises approximately 80 per cent. of the total protein of milk, is believed to be present in combination with calcium, and is not in solution but in the form of very small gelatinous suspended particles. By the action of dilute acids or of rennet the free casein is precipitated. Free casein is readily soluble in dilute alkalies, from which it is precipitated by adding acid. Its alkaline solutions are levo-rotatory, having a specific rotation of about $-90°$. The soluble albumin (lactalbumin) makes up by far the greater part of the remaining protein and is quite similar to the albumin of egg, being readily soluble in water, coagulated by heating to 75°C., and having a specific rotation of about $-68°$. The mineral salts existing in milk are to a certain extent conjectural, since the salts found by analysis of the ash are not exactly the same as those present in the milk itself. They consist largely, however, of the chlorides, phosphates and citrates of sodium, potassium, calcium and magnesium. Of the other substances which occur in milk, mostly in very small amounts, citric acid, which is present up to 0.15 per cent. in the form of citrates, is the only one which need be mentioned.

Composition

General Composition.—In approximate figures the average percentage composition of milk may be stated:

	Per cent.
Total solids	12.8
Fat	3.8
Protein	3.6
Ash	0.7
Milk sugar	4.7
Solids not fat	9.0

From these figures there may be in normal milk quite decided variations and figures have been reported which differ widely from them, some of the discrepancies of the older analyses being undoubtedly due to the imperfect methods of analysis employed.

Lythgoe[1] states that all milk completely drawn from healthy cows will fall between the following limits:

TABLE X

	Extreme limits, per cent.	Usual limits, per cent.	Herd milk, per cent.
Total solids	10.0–17.0	10.5–16.0	11.8–15.0
Fat	2.2– 9.0	2.8– 7.0	3.2– 6.0
Protein	2.1– 8.5	2.5 –4.5	2.5– 4.0
Ash	0.6– 0.9	0.7– 0.8	0.7– 0.8
Milk sugar	4.0– 6.0	4.2– 5.5	4.3– 5.3
Solids not fat	7.5–11.0	7.7–10.0	8.0– 9.5

Variations in Composition.—Besides variations in composition which may be due to individual cows, there are also certain well established differences due to environment or to racial influences. Among the more important of these are:

1. *The Breed of the Cow.*—Some breeds yield quantity, others quality. The Jersey and Guernsey cattle, for instance, give comparatively small quantities of milk rich in fat; the Holstein cows, on the other hand, yield much larger amounts of milk of decidedly lower solids and fat content. These differences are well summarized in the following table based on data collected by the Massachusetts Board of Health.[2]

If individual differences are eliminated and only fully drawn mixed milk from herds is considered, the variation due to breed

[1] *Bull. Mass. State Bd. Health*, **1910**, p. 419.
[2] *Bur. of Chem., Bull.* **132**, p. 129.

TABLE XI

Breed	Specific gravity	Total solids, per cent.	Fat, per cent.	Protein, per cent.	Ash, per cent.	Solids not fat, per cent.	Milk sugar, per cent.
Jersey	1.034	14.57	5.40	3.54	0.78	9.17	4.85
Guernsey	1.034	14.40	5.00	3.77	0.77	9.40	4.86
Ayrshire	1.032	12.57	4.00	2.90	0.77	8.57	4.90
Dutch Belt	1.032	12.03	3.60	2.62	0.68	8.43	5.00
Holstein	1.032	11.96	3.35	2.99	0.69	8.61	4.89

is the factor of the greatest influence in permanently affecting the composition of milk.

2. *The Time of Year.*—The poorest milk is produced during the spring and early summer months, the richest during the seasons of autumn and early winter, when the cattle are getting a smaller proportion of green feed. This difference is clearly shown in the following table[1] which gives the seasonal average for 16 years:

	Total solids, per cent.	Fat, per cent.	Solids not fat, per cent.
Nov.–Jan.	13.04	4.11	8.93
Feb.–Apr.	12.72	3.88	8.84
May –Aug.	12.66	3.89	8.77
Oct.–Nov.	13.03	4.25	8.78

This variation in composition of milk between the pasture-fed and the stall-fed season has in the past received legal recognition in the fixing of milk standards. In Massachusetts for many years the legal standard for total solids was set at 13 per cent. in the winter months and at 12 per cent. in the summer season.

3. *Time of Day.*—Milk which has been drawn in the evening is nearly always richer in fat than the morning milk, as shown in the following averages:

	Specific gravity	Total solids	Fat
Morning milk	1.0322	12.53	3.63
Evening milk	1.0318	12.94	4.04

[1] Richmond: Dairy Chemistry, p. 126.

4. *"Fore" Milk vs. "Strippings."*—If different portions of the whole quantity of milk obtained at a single milking are examined separately they will be found to show marked differences in fat content, especially as between the first and last portions. The other constituents of the milk do not vary so greatly as the fat. The first portions of milk, the "fore" milk, contain much less fat than do the last portions or "strippings." The following figures, due to Van Slyke, illustrate this point:

	Per cent. of fat in milk		
	Cow 1	Cow 2	Cow 3
First portion drawn	0.90	1.60	1.60
Second portion drawn	2.60	3.20	3.25
Third portion drawn	5.35	4.10	5.00
Fourth portion drawn (strippings)	9.80	8.10	8.30

This difference in composition is explained by the separation of the milk while in the udder of the cow, cream rising to the top just as would happen if the milk stood in a vessel, hence being drawn last. Dishonest dairymen have in the past taken advantage of this fact in adulteration cases by having the cows *partially* milked in the presence of unsuspecting witnesses, the resulting "known purity" milk being thus largely "fore" milk.

In general it will be found that to whatever causes the variations noted in the composition of milk are due, the differences are shown much more in the fat than in any other constituent. The protein is also variable, although to a somewhat less extent, and the milk sugar and ash are much more nearly constant.

Methods of Analysis

Preparation of the Sample.—Since the cream will rise on a sample of milk sufficiently in 5 minutes to destroy the uniformity of the sample, great care must be used in taking a portion for analysis to ensure that it represents a fair average of the milk. The best way is to pour the milk from the containing vessel into another and back again several times, or if this is impracticable it should be thoroughly stirred before being sampled. If

the analytical sample has stood for any appreciable time it should be mixed by pouring back and forth before a portion is removed to test, otherwise concordant results cannot be obtained. Do not shake the sample, since this tends toward a separation of the fat.

Specific Gravity.—This is usually taken with a special form of hydrometer known as a *lactometer* (Fig. 38). The Quevenne lactometer has a scale graduated into twenty-five equal parts, extending from 15 to 40, corresponding to specific gravities from 1.015 to 1.040. The best form of instrument is that provided with a thermometer.

The lactometer is graduated to give correct results at 60°F. (15.6°C.) and the reading should be made at approximately that temperature, between 55° and 65°, and then corrected to standard temperature. This may be done by adding 0.1 to the reading for each degree F. above 60°F., or subtracting 0.1 for each degree F. below 60°F. If the temperature is read in Centigrade degrees the correction may be made by the following table:

TABLE XII.—FOR CORRECTING THE SPECIFIC GRAVITY OF MILK ACCORDING TO TEMPERATURE. ADAPTED FROM THE TABLE OF VIETH

(Temperature in Degrees Centigrade)

Specific gravity	10°	11°	12°	13°	14°	15°	16°	17°	18°	19°	20°
1.025	24.1	24.3	24.5	24.6	24.7	24.9	25.1	25.3	25.4	25.6	25.9
26	25.1	25.2	25.4	25.5	25.7	25.9	26.1	26.3	26.5	26.7	27.0
27	26.1	26.2	26.4	26.5	26.7	26.9	27.1	27.4	27.5	27.7	28.0
28	27.0	27.2	27.4	27.5	27.7	27.9	28.1	28.4	28.5	28.7	29.0
29	28.0	28.2	28.4	28.5	28.7	28.9	29.1	29.4	29.5	29.8	30.1
30	29.0	29.1	29.3	29.5	29.7	29.9	30.1	30.4	30.5	30.8	31.1
31	29.9	30.1	30.3	30.4	30.6	30.9	31.2	31.4	31.5	31.8	32.2
32	30.9	31.1	31.3	31.4	31.6	31.9	32.2	32.4	32.6	32.9	33.2
33	31.8	32.0	32.3	32.4	32.6	32.9	33.2	33.4	33.6	33.9	34.2
34	32.7	33.0	33.2	33.4	33.6	33.9	34.2	34.4	34.6	34.9	35.2
35	33.6	33.9	34.1	34.4	34.6	34.9	35.2	35.4	35.6	35.9	36.2

Directions.—Find the observed gravity in the left-hand column. Then, in the same line, and under the observed temperature will be found the corrected reading.

The New York Board of Health lactometer has a scale reading 0 in water, and 100 in milk with a specific gravity of 1.029, which is taken as the lowest limit for pure milk. The instru-

ment is used in the same manner as the Quevenne lactometer and the readings can be converted into degrees of the latter instrument by multiplying by 0.29.

Notes.—The specific gravity of milk fat is about 0.93; of the solids not fat approximately 1.5. The specific gravity of the milk itself is thus a function of the two; the former lowers it, the latter increases it. As would be expected from the variable composition of milk, the specific gravity is also a variable. The values for normal milk from a herd, however, will usually fall between 1.030 and 1.034.

Taken by itself the specific gravity is of little value in showing adulteration. The addition of water lowers the specific gravity of milk; the removal of cream raises it, this being the lighter portion of the milk. It is therefore theoretically possible by skilful manipulation to both skim and water a sample and still have its specific gravity correspond to that of normal milk. Such a sample would, however, be readily recognized by one familiar with the appearance of the genuine product.

The lactometer reading is of value in the rapid analysis of milk for calculating the solids in connection with the Babcock method of fat determination. (See page 119.)



Fig. 38.—Lactometers.

Total Solids.—Use a platinum dish having a flat bottom about 2½ in. in diameter. Ignite and weigh the dish accurately, then add about 5.1 grams to the weights on the balance-pan. With a pipette deliver 5 cc. of the well-mixed milk into the dish and weigh the whole as rapidly as possible to the nearest milligram. Evaporate the milk to dryness on the water-bath and then dry it in the oven at 100°C. to constant weight. Three hours drying is usually sufficient.

Notes.—It is important that the milk should be dried in a thin layer, so that the removal of the water shall take place as quickly as possible. Under these conditions the residue obtained is

nearly white, but if the process be prolonged, it may have a brownish color from the caramelization of the sugar.

If it is not desired to determine ash on the same weighed portion as used for the solids, lead foil dishes or tin blacking box covers may be used instead of platinum dishes.

For the determination of the total solids by calculation from the fat and lactometer reading see page 119.

Ash.—Ignite the platinum dish containing the residue from the preceding determination at a low red heat until the ash is white or of a uniform light gray color. This may be done in a muffle furnace at a temperature not exceeding 600°C., or over a burner carefully regulated so that the dish is nowhere heated above the slightest visible redness.

The ash, after weighing, may be tested for boric acid as described on page 98.

Fat.—(*a*) *Adams' Paper Coil Method.*—Roll a strip of fat-free blotting paper[1] about 22 in. long and 2½ in. wide, into a loose coil and fasten it by a bit of wire. Hold the coil in one hand and slowly run on to the upper end of it exactly 5 cc. of milk from a pipette. If preferred, about 5 grams of milk may be weighed quickly in a small beaker, and one end of the coil introduced so as to absorb the milk, care being taken to absorb it as nearly completely as possible. The beaker is then quickly re-weighed.

Place the coil, after charging with the milk, dry end downward, in the water oven and dry it for two hours, then extract it for at least 2 hours in a Soxhlet extractor as described on page 22 using either petroleum ether or anhydrous ethyl ether. At the end of this time disconnect the apparatus when the extractor is nearly full of ether, thus recovering a large portion of the solvent, and evaporate the remainder away from a flame, as described on page 24. Dry the fat to constant weight in the water-oven. In drying the extracted fat it may be heated for 2 hours the first time, then in 1 hour periods until the loss of weight is not over a milligram.

Notes.—The only part of the method due to Adams is the dry-

[1] Schleicher and Schüll make suitable strips which can be obtained from dealers in chemical supplies, or the strips may be previously prepared in the laboratory from thick filter paper and extracted with ether before using.

ing of the milk on porous paper. This is, however, of great importance, since the absorbent paper exercises a selective action on the constituents of milk so that the fat is left on the surface of the paper, mixed with only about one-third of the non-fatty solids, and hence is more easily extracted; further, owing to the greatly increased surface exposed, the extraction of the fat is practically complete in a comparatively short time.

Ethyl ether is the solvent commonly employed, but care should be taken that it is anhydrous, otherwise small amounts of milk sugar will be extracted. For this reason petroleum ether is to be preferred as a solvent, although its action is considerably slower than that of the other.

The Adams method is probably the most accurate for fat determination in milk, but in actual practice is not used so much as the more rapid centrifugal methods.

(*b*) *Babcock Method.*—Measure 17.6 cc. of the milk from a pipette into the graduated test bottle; add 17.5 cc. of sulphuric acid (sp. gr. 1.825) pouring it in slowly so as to form a layer beneath the milk. After the acid has thus been added to all the bottles mix the milk and acid thoroughly by a rotary motion, avoiding the spurting of the liquid into the neck of the bottle. Place the bottles in opposite pockets of the centrifuge, in even numbers, and whirl them for 5 minutes at the proper speed. The correct speed varies from 1000 revolutions per minute for a 10-in. wheel to 700 for one of 24 in. diameter. Then remove the bottles and add hot water up to the necks, after which whirl them again for 1 minute. Again add hot water until the fat rises nearly to the top of the graduations. Whirl again for 1 minute. Then measure the length of the column of fat by a pair of dividers, the points being placed at the extreme limits of the column, the fat being kept warm, if necessary, by standing the bottles in water at 60°C. If now one point of the dividers is placed at the 0 mark of the scale on the bottle used, the other will indicate the per cent. of fat in the milk.

Notes.—Methods based on centrifugal separation of the fat, of which the Babcock method is the pioneer, are by far the most rapid and convenient for general use. They have practically replaced the more tedious extraction methods and are universally employed in creameries and milk depots.

When the acid and milk are mixed the mixture becomes hot and turns dark colored on account of the charring of the milk sugar. The casein is first precipitated and then dissolved. The retarding effect of the milk serum solids being thus eliminated, the fat globules are free to collect in a mass.

The fat obtained should be of a clear, golden yellow color, and distinctly separated from the acid solution beneath it. If the fat is light-colored or whitish, often with a layer of white particles beneath it, it generally indicates that the acid is too weak or that the milk was too cold when the acid was added. A dark-colored fat with a sub-stratum of black particles indicates that the acid is too strong. The best results will be obtained by the use of acid of the strength noted above.

The capacity of the graduated neck of the bottle between the 0 and 10 marks is 2 cc. The specific gravity of warm milk fat is 0.9, hence 2 cc. will weigh 1.8 grams or one-tenth of the weight of 17.6 cc. of milk (approximately 18 grams). The measurement to the extreme limits of the column of fat, rather than to the upper meniscus, is to correct for the small amount of fat, 0.1 to 0.2 per cent., that remains in the acid solution.

Milk which has been preserved with formaldehyde usually requires a longer time and more vigorous shaking to dissolve the curd, on account of the hardening action of this preservative on the coagulated casein. It is often advantageous to stand the bottles in water at 60°C. for a time before whirling. Samples containing formaldehyde will usually give a violet color when the acid is added to the milk. (See page 89.)

(c) *Gottlieb Method.*[1]—With a pipette place 5 cc. of milk in a 50-cc. glass-stoppered cylinder and add the following reagents, being careful to add them in the order given and to shake the stoppered cylinder thoroughly after the addition of each reagent: 1 cc. of ammonia (sp. gr. 0.96), 5 cc. of alcohol, 12.5 cc. of ethyl ether and 12.5 cc. of petroleum ether. Let the cylinder stand until the lower layer is free from bubbles, several hours if necessary. Transfer the upper layer to a tared flask by means of an arrangement similar to a wash-bottle as shown in Fig. 39. Adjust the sliding tube until the end rests just above the junction of the two layers, then by gently blow-

[1] Röse: *Z. angew. Chem.*, **1888**, 100; Gottlieb: *Landw. Vers. Stat.*, **1892**, 6.

ing force out the upper layer into the flask. Repeat the extraction, using 10 cc. each of ethyl ether and petroleum ether and blowing it off into the flask as before. Distil off the solvent and dry the residual fat to constant weight in the water-oven. Dissolve the weighed fat in a little petroleum ether. If a residue is found, due to a trace of the aqueous layer which was blown off with the ether, wash it several times in the flask by careful decantation with petroleum ether. Finally dry and weigh the flask and residue and deduct from the previous weight. The difference is the weight of purified fat.

Notes.—All of the successful methods for determining the fat by direct extraction from the milk itself involve the complete or partial solution of the casein. In the Gottlieb method the casein, precipitated from the milk in very finely divided form by the alcohol, is dissolved by the ammonia. The fat is dissolved by the ethyl ether and the addition of petroleum ether is to render less soluble the milk sugar or other non-fatty solids which would be dissolved by ethyl ether alone.

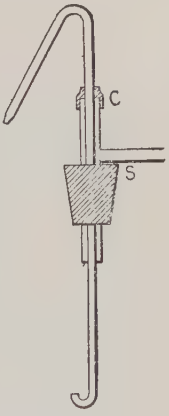


FIG. 39.

The method, while applicable to whole milk, is especially valuable in determining fat in such products as skim milk or buttermilk, which are low in fat. In such cases it is better to use 10 cc. of milk and double the quantity of reagents.

Milk Sugar.—The sugar in milk can be determined either by means of the polariscope or by its reducing action on Fehling's solution.

(*a*) **Optical Determination.**—*Method of Double Dilution.*[1]—Into each of two flasks graduated at 100 and 200 cc. respectively, place 65.8 grams of milk, add 5 cc. of acid mercuric nitrate,[2] fill to the mark and mix by shaking. Filter through dry filters and polarize in a 400-mm. tube, using the Schmidt and Haensch or similar saccharimeter.

Calculation of Results.—The actual reading in each case is

[1] Wiley and Ewell: *Analyst*, **1896**, 182.

[2] Dissolve mercury in twice its weight of nitric acid (sq. gr. 1.42) and add to the solution an equal volume of water.

too high on account of the volume occupied by the precipitated proteins and fat. Since this is the same in the two flasks, however, the error is only one-half as great with the larger dilution. The method of calculation, based on this fact, can be seen from the following example:

Weight of milk used	= 65.8 grams
Reading from 100-cc. flask	= 20.84 (400-mm. tube)
Reading from 200-cc. flask	= 10.15 (400-mm. tube).

Then if no correction for precipitate were to be applied the reading from the 100-cc. flask should be

$$10.15 \times 2 = 20.30$$

Hence $20.84 - 20.30 = 0.54$, the error due to volume of precipitate in the 200-cc. flask.

Therefore, $0.54 \times 2 = 1.08$; the correction due to volume of precipitate in the 100-cc. flask.

$20.84 - 1.08 = 19.76$, the true reading from 100-cc. flask. Since twice the normal weight and twice the standard tube length were used, $19.76 \div 4 = 4.94$, the per cent. of milk sugar.

Notes.—The normal weight of lactose for saccharimeters with the Ventzke scale can be readily calculated from the sucrose normal weight (see page 253) by the inverse ratio of their specific rotations. Thus

Lactose		Sucrose
52.5	:	66.5 = 26 grams: x, whence x = 32.9 grams.

Since the percentage of lactose in milk is relatively small it is better to employ double the normal weight and the longer tube.

Basic lead acetate, the clarifier ordinarily used in sugar polarizations, is not suitable in the case of milk since it has been shown by Wiley[1] that it leaves notable quantities of levorotatory substances still in solution.

For less exact work the method may be materially shortened by employing a constant correction of 2.6 cc. for the volume of the precipitate, or if the percentages of fat and protein are known their respective volumes can be calculated from the

[1] *Am. Chem. J.*, **1884**, 289.

specific gravity.[1] Thus, since the specific gravity of milk fat is 0.93, the volume of precipitated fat may be found by dividing the grams of fat by 0.93. Similarly the volume of casein may be found by dividing its weight by 1.25. The sum of the two is the volume of the precipitate.

The optical determination of milk sugar requires extremely careful readings on account of the dilute solution employed. For this reason, the copper reduction method, as described below, is preferable in most cases.

(*b*) **Determination by Fehling's Solution.**—Measure 25 cc. of milk into a 500-cc. graduated flask. Add about 400 cc. of water, 10 cc. of copper sulphate solution,[2] then 35 cc. of tenth-normal sodium hydroxide (or an equivalent quantity of a stronger solution) and make up to 500 cc. Mix thoroughly and filter through a dry filter. Use 50 cc. of the filtrate for the determination of the lactose by the Munson and Walker method as described on page 237. Express the result as per cent. of lactose monohydrate, calculating the weight of 25 cc. of the milk from its specific gravity.

Notes.—Before the lactose can be determined by Fehling's solution the proteins and fat must first be removed. This is done by the precipitation with copper hydroxide, the fat being carried down mechanically by the precipitated proteins. The addition of alkali should be such that a slight excess of copper still remains in solution, since an excess of alkali will prevent the precipitation of part of the protein. The quantity stated in the procedure is correct for most milks.

On account of the considerable dilution of the sample the volume of the precipitated protein and fat need not be considered.

Proteins.—Determination of Total Protein.—This is best done by the Kjeldahl method. Weigh 5 grams of milk into a Kjeldahl flask, add 10 cc. of concentrated sulphuric acid and 3 drops of mercury and carry out the determination as described on page 25.

The tendency of the alkaline solution to froth during the

[1] Leffman and Beam: Food Analysis, p. 212.

[2] 69.28 grams per liter. The copper sulphate solution used in the Fehling mixture may be conveniently employed.

distillation, which is especially noticeable with milk, can be prevented by the addition of a piece of paraffin the size of a pea. Multiply the per cent. of nitrogen by the factor 6.38 to obtain the per cent. of protein.

Separation of Casein and Albumin.—Casein can be separated from albumin by precipitation with acid at a temperature below the coagulating point of the albumin. The method adopted by the Official Agricultural Chemists[1] is practically that worked out by Van Slyke and Hart.[2]

Casein.—To 10 grams of milk add 90 cc. of water at 40–42°C. and then 1.5 cc. of 10 per cent. (by weight) acetic acid. Shake and maintain at the stated temperature until a flocculent precipitate separates, leaving a clear supernatant liquid. Filter, wash, and determine the nitrogen in the washed precipitate and filter by the Kjeldahl method. Multiply by 6.38 for the casein.

Albumin.—To determine the albumin, neutralize the nitrate with caustic alkali and phenolphthalein and heat it at 100°C. until the precipitate settles clear. Filter, wash and determine the nitrogen as above. Nitrogen multiplied by 6.38 equals albumin.

Note.—The above methods, while capable of good results, are tedious and rather unsatisfactory except in the hands of chemists of considerable experience. The following volumetric method, devised by Van Slyke and Bosworth[3] gives results of almost equal accuracy but requires much less time and skill:

Measure 20 cc. of the well-mixed milk into a 200-cc. graduated flask and add about 80 cc. of water. Add 1 cc. of phenolphthalein solution and tenth-normal sodium hydroxide until a faint pink color remains throughout the mixture even after considerable shaking. Avoid an excess of alkali.

To the neutralized diluted sample, which should be at a temperature of 18°C. to 24°C., add tenth-normal acetic acid from a burette in 5-cc. portions, shaking vigorously for a few seconds after each addition. After thus adding 25 cc. and shaking, the mixture is allowed to come to rest. If enough acid has been added, the casein separates promptly in large, white flakes, and on

[1] *Bur. of Chem., Bull.* **107**, p. 117.

[2] *J. Am. Chem. Soc.*, **1893**, 635; *Am. Chem. J.*, **1903**, 170.

[3] *J. Ind. Eng. Chem.*, **1909**, 768.

standing a short time the supernatant liquid appears clear, not at all milky. If the addition of 25 cc. of acid is insufficient to separate the casein properly, add 1 cc. more of acid and shake; continue this addition of acid 1 cc. at a time, until the casein separates promptly and completely upon standing a short time. Note the number of cc. of acid used.

After the casein is completely precipitated make up the mixture to the 200-cc. mark with water, shake thoroughly and filter through a dry filter. Filtration should be rapid and the filtrate quite clear. If marked turbidity is apparent in the filtrate, a new sample should be taken and the process repeated, using more acid than before. Titrate 100 cc. of the filtrate with tenth-normal sodium hydroxide and phenolphthalein to a pink color which remains throughout the solution for 30 seconds. Subtracting the number of cc. of sodium hydroxide from one-half the cc. of tenth-normal acetic acid added will give the cc. of acid required to precipitate the casin from 10 cc. of milk. (1 cc. of $\frac{N}{10}$ acetic acid = 0.11315 grams of casein.)

Calculations of Milk Solids.—It has long been recognized that in normal milk the constituents are present in a fairly constant ratio. This being true, it should be possible, having determined two factors, to find a third by calculation, or at least to show by such calculation a sufficient variation from the normal to indicate the adulteration of the sample. For example, given the lactometer reading and fat, to calculate the total solids:

L = the lactometer reading.
s = increase in lactometer reading by 1 per cent. solids not fat.
f = decrease in lactometer reading by 1 per cent. fat.
T = total solids.
S = per cent. of solids not fat.
F = per cent. of fat.

Then $L = Ss - Ff$.
Since $S = T - F$.
$L = (T - F)s - Ff$
whence

$$T = \frac{L \times Ff}{s} + F$$

The uncertainty of the calculation lies in the values for s and f, which on account of the difference in solution densities of the components of the solids not fat are not absolute constants.

Based on the principle just stated various formulæ have been proposed for the calculation of milk solids. One of the simplest of these is that of Hehner and Richmond.[1]

$$T = \frac{L}{4} + 1.2\,F + 0.14$$

when T is the per cent. of total solids, L the reading of the lactometer and F the fat.

When a number of calculations are to be made, Richmond's "Milk Scale" (Fig. 40) will be found convenient. This is an instrument based on the principles of the slide-rule, having three scales, two of which, for the fat and the total solids, are marked



FIG. 40.—Richmond's milk scale.

on the body of the rule, while that for the lactometer readings is marked on the sliding part.

A similar relation has been worked out for the proteins, so that if a constant value be assumed for the ash the composition of a sample may be determined with a fair degree of approximation from the two simple determinations of specific gravity and Babcock test.

The relation between the proteins and fat has been expressed by Van Slyke[2] as $P = 0.4\ (F - 3) + 2.8$. Similarly Olson[3] has proposed the following formula for calculating the protein from the total solids (T. S.):

$$P = T.S. - \frac{T.S.}{1.34}$$

These values will naturally be most nearly correct in the case of normal average milk. With watered or skimmed milk they will be only approximate. An illustration of the use of the above formulæ in detecting adulteration is given on page 134.

[1] *Analyst*, **1888**, 26; **1892**, 170.
[2] *J. Am. Chem. Soc.*, **1908**, 1182.
[3] *J. Ind. Eng. Chem.*, **1909**, 253.

In the table below the calculated values on a sample are compared with those actually determined:

Determination	Actual values	Calculated values
Lactometer reading	33.0	
Fat (Babcock)	3.80	
Total solids	12.73	12.95
Ash	0.71	0.7 (assumed)
Proteins	3.33	3.12 (Van Slyke) 3.29 (Olson)
Milk sugar	5.04	5.16
Solids not fat	8.93	9.15

Examination of Milk Serum.—The most variable constituents of normal milk are the fat and proteins, especially the former; the least variable are the ash and milk sugar. The milk serum, or milk from which the fat and proteins have been removed, is therefore of more uniform composition than the milk itself, hence better suited for the detection of adulteration and especially of added water. The serum may be prepared by adding to the milk some suitable precipitant of the proteins, as calcium chloride, acetic acid or copper sulphate. The clear liquid after filtration may be examined for its content of dissolved solids, its specific gravity or most conveniently by the immersion refractometer. The calcium chloride method[1] is largely employed in Germany but most of the data available on American milks has been obtained by the use of the other two methods.

(*a*) *The Acetic Acid Method.*[2]—To 100 cc. of the milk at a temperature of about 20°C. add 2 cc. of 25 per cent. acetic acid (sp. gr. 1.035) in a beaker, cover with a watch-glass and heat in a water-bath at 70°C. for 20 minutes. At the end of that time place the beaker in ice water for 10 minutes, then filter the solution. With the clear filtrate determine either the refraction at 20°C. using the immersion refractometer as described on page 11, or the specific gravity at 15°C. using a test-tube or other suitable narrow cylinder and the Westphal balance. (See page 1.)

[1] Ackermann: *Z. Nahr. Genussm.*, **1906,** 405; **1907,** 186.

[2] Woodman: *J. Am. Chem. Soc.*, **1899,** 503; Leach and Lythgoe: *J. Am. Chem. Soc.*, **1904,** 1195.

Note.—With normal milk, free from added water, the specific gravity of the acetic acid serum is ordinarily above 1.0270 at 15°C. and the refractometer reading above 39° at 20°C. The lowering of the specific gravity is about 0.0031 and the lowering of the refraction about 2.7 for every 10 per cent. of added water.

(*b*) *The Copper Sulphate Method.*[1]—Dissolve 72.5 grams of crystallized copper sulphate in water and dilute to a liter. This solution should be adjusted, if necessary, so that it will refract at 36° on the scale of the immersion refractometer at 20°C., or have a specific gravity of 1.0443 at 20°C., compared with water at 4°C. To one volume of the copper solution add four volumes of milk, shake well and filter. The filtrate will usually be clear after the first few drops have passed through. On the clear filtrate either the refraction at 20°C., the specific gravity at $\frac{20°}{4°}$C. or the total solids may be determined.

Notes.—The copper sulphate method is preferable to the use of acetic acid in that it is quicker, there is no heating with consequent chance for loss by evaporation, and the variation in results is less in the case of pure milk. Ten per cent. of added water will show by the decreased refraction, when not less than 15 per cent. would be detected by the acetic acid method.

Examination of the copper serum from 150 samples of known purity milk gave refractions varying from 36.1 to 39.5, while the total solids of the same samples showed a range from 17.37 to 10.40 per cent. and the fat varied from 7.7 to 2.45 per cent., which is sufficient evidence of the value of the method.

The minimum values for the copper serum of normal milk are 36° for the refraction at 20°C., 1.0245 for the specific gravity at $\frac{20°}{4°}$C. and 5.28 per cent. for total solids.

If the milk is already soured, it may be filtered and similar determinations made on the natural sour serum, which for unwatered milk should not refract below 38.3 or have a specific gravity at $\frac{20°}{4°}$C. below 1.0229.

It will be noticed that the refraction of the sour serum is midway between that of the copper sulphate serum and the acetic

[1] Lythgoe: *Ann. Rept. Mass. Bd. Health*, **1908**, p. 594.

acid serum, a fact which should be borne in mind if the sample of milk has become partly sour before being analyzed. Under such circumstances, the examination of the serum would best be confined to the spontaneously obtained sour serum.

The greatest value of the sour serum, however, is for the determination of ash, which may be done by evaporating 25 cc. of it to dryness in a platinum dish and igniting in a muffle at a temperature not above 600°C. In unwatered milk the ash content of the sour serum should not be less than 0.73 per cent. The special value of the determination, as pointed out by Lythgoe,[1] lies in the fact that there is no relation between the refraction of the serum and the ash of the sour serum; hence, if both results are below the minimum for pure milk, it is positive indication of the presence of added water.

Special Tests for Adulterants

Cane Sugar.—Cane sugar may be present in milk from diluted condensed milk used to eke out the supply, or may be present from calcium saccharate, added as a thickening agent (see page 140, under Cream). It is evident that any considerable amount which had been added would be detected by the taste.

To detect the presence of cane sugar boil about 10 cc. of the milk with 0.1 gram of resorcinol and 1 cc. of strong hydrochloric acid for 5 minutes. The liquid will be colored rose red if cane sugar is present. The color produced by heating should not be confused with the pink color which may appear in the cold if the milk contain certain coal-tar colors. The test is more fully described on page 236.

A similar test is the reduction of ammonium molybdate. As recommended by Cotton,[2] 10 cc. of the milk are mixed with 0.5 gram of powdered ammonium molybdate and 10 cc. of dilute (1 to 10) hydrochloric acid are added. In another tube 10 cc. of milk known to be free from sucrose are similarly treated and the tubes placed in a water-bath, the temperature of which is gradually raised to about 80°C. If sucrose is present, the milk remains unchanged unless the temperature approaches the boiling point.

[1] *J. Ind. Eng. Chem.*, **1914**, 899.
[2] *J. Pharm. Chim.*, **1897**, 362.

Cotton states that the reaction will detect as little as 1 gram of cane sugar in a liter of milk.

The ammonium molybdate test in the form used for detecting calcium saccharate in cream (see page 144) may also be used to show the presence of cane sugar in milk.

Note.—As with the resorcinol test, the molybdate reaction is given by levulose as well as by sucrose.

Gelatin and Calcium Sucrate.—These substances, although possibly used to thicken milk, are more commonly found in cream and their detection is described on pages 141 and 144.

Preservatives.—The preservatives most commonly employed in milk are formaldehyde, boric acid or borax, and mixtures of the two, and possibly hydrogen peroxide and fluorides. Salicylic acid and sodium benzoate, although largely used in some other classes of food materials, have been practically never reported as present in milk.

Formaldehyde.—This is the ideal preservative for milk, being readily used and by far the most efficient. Quantities which give a proportion in the milk of from 1 in 10,000 parts to 1 in 50,000 are ordinarily employed. Such an amount will suffice to preserve the milk from 24 hours to several days. Larger quantities, such as 1 part in 3,000, will preserve the milk for months. These large amounts, however, would be more or less apparent by the taste or odor. A tabular statement showing the efficiency of formaldehyde in preserving milk as compared with boric acid, borax and sodium carbonate will be found in Leach's Food Analysis.

Several of the best tests for detecting formaldehyde are described on page 89, in the chapter on Preservatives. These may be applied directly to 10 cc. of the milk, or as suggested in the gallic acid test, a larger quantity, 25 to 100 cc., may be distilled and the test applied to the first portion of the distillate.

It should be borne in mind that when small amounts of formaldehyde are added to milk the ordinary tests will show the presence of the preservative for only a short time. For example it has been shown by Williams and Sherman[1] that when formaldehyde was added to milk in the proportion of 1 part to 100,000 only a faint test was given after 48 hours standing and the

[1] *J. Am. Chem. Soc.*, **1905**, 1497.

preservative had entirely disappeared in from 3 to 5 days. This is due to the gradual formation of condensation products of the formaldehyde with the proteins of the milk which do not respond to the usual reactions. In such a case it is better to distil the milk as directed and apply the gallic acid test to the distillate. The test is thus made more delicate, so that the preservative may still be shown when the simpler tests have failed.

Another possible contingency is that some substance may be added with the formaldehyde which will interfere with the tests for its detection. Both hydrogen peroxide and nitrites prevent the reaction of formaldehyde in the usual tests, and preservatives are on the market which are mixtures of formaldehyde with hydrogen peroxide or a nitrite. Hehner's test and the hydrochloric acid-ferric chloride test (page 89) can be used to show the formaldehyde in the presence of considerably larger quantities of nitrite or hydrogen peroxide by previous treatment with urea. Add to 10 cc. of the milk 1 cc. of a 10 per cent. solution of urea, then 2 cc. of dilute (1:40) sulphuric acid and immerse the test-tube in boiling water for 2 minutes. Cool and carry out the test as usual. The reactions between the urea and the nitrous acid or hydrogen peroxide may be expressed:

$$CO(NH_2)_2 + 2HNO_2 \rightarrow CO_2 + 2N_2 + 3H_2O$$
$$CO(NH_2)_2 + 3H_2O_2 \rightarrow CO_2 + N_2 + 5H_2O$$

The phenylhydrazine test for formaldehyde,[1] although not so well suited for general use as the three described on page 89, has the advantage that it may be used in the presence of hydrogen peroxide or nitrites. This method is as follows: To 10 cc. of the milk add about 0.1 gram of phenylhydrazine hydrochloride, then 2 drops of a freshly prepared 10 per cent. solution of sodium nitroprusside and finally 10–12 drops of 10 per cent. sodium hydroxide. In the presence of formaldehyde a blue-green color is formed which after a long time changes to red.

Hydrogen Peroxide.—This has been reported as being used to some extent as a milk preservative, but its value is slight since the small amount added rapidly disappears in the presence of

[1] Rimini: *Ann. di Farmacol.*, **1898**, 97; abs. *Chem. Zentr.*, **1898**, I, 1152.

the organic matter. For this reason any tests which will show its presence must be extremely delicate and be applied to the fresh milk.

The most delicate test is by means of *p*-phenylenediamine. To 10 cc. of the milk add 3 drops of a freshly prepared 2 per cent. aqueous solution of *p*-phenylenediamine hydrochloride and shake. The presence of hydrogen peroxide is indicated by the gradual appearance of a deep blue color.

Note.—The reaction is dependent upon the presence in fresh milk of an oxidase or enzyme which has the power of bringing about the transfer of oxygen from peroxides to oxidizable substances. Since this enzyme is destroyed by heating, as explained more fully on page 128, it is necessary that the test be made on uncooked milk. If it is desired to test for peroxides in cooked milk, as in pasteurized milk, an equal volume of unheated milk, known to be free from peroxide, should first be added.

Boric Acid and Borax.—Use from 25 to 100 cc. of the sample and proceed as directed on page 98.

Salicylic and Benzoic Acids.—If it is desired to test for these, the following method may be employed: To 25 cc. of milk add 100 cc. of water and precipitate the proteins and fat with copper sulphate and sodium hydroxide, as described on page 117. Filter and add to the filtrate 5 cc. of concentrated hydrochloric acid. Extract with ether and proceed with the qualitative tests for benzoic and salicylic acids as outlined on page 90, *et seq.*

Fluorides.—To 100 cc. of milk add an equal volume of water, heat to boiling and proceed with the method as given on page 101.

Coloring Matter.—The object in adding coloring matter to milk is in general to disguise the bluish appearance of skimmed or watered milk. For this reason it is rather unusual to find added color in the case of milk which is of standard quality, although such cases have been reported.

Formerly the chief color used was annatto, a reddish-yellow coloring matter obtained from the seeds of *Bixa Orellana*, a shrub growing in South America and the West Indies. A solution of the color in very dilute alkali is employed. More recently various coal-tar dyes and even caramel have been used. The latter is perhaps not so likely to be found because its color is too brown and not enough yellow to give the desired creamy appear-

ance to the milk, which is so easily obtained with annatto. The coal-tar colors, especially mixtures of yellow and orange azo dyes, give very good results.

Leach[1] has suggested a general scheme for the identification of these colors in milk, which with some modifications which experience in the writer's laboratory has shown helpful in detecting annatto especially, is given below.

Procedure.—Place about 100 cc. of the milk in a small beaker, add 3–4 cc. of 25 per cent. acetic acid (sp. gr. 1.04), stir thoroughly and allow the beaker to stand quietly on the water-bath for about 10 minutes, the casein being thus separated as a compact cake. Decant off the whey, squeezing the curd as dry as possible with a spatula. Transfer the curd to a flask, cover it with ether, stopper tightly, and shake the flask violently in order to break up the curd as much as possible. Let it stand for several hours, preferably over night.

Pour off the ether, which contains the annatto, and evaporate (*away from a flame!*) until no odor of ether remains. Add 5 cc. of water and then dilute sodium hydroxide until the mixture, after thorough stirring with a glass rod, is faintly alkaline to litmus paper, and filter through a wet filter. If annatto is present it will permeate the filter and give it an orange-brown color which may readily be seen if the filter is removed from the funnel and the fat washed off under the tap. Its presence may be confirmed by touching the colored portion of the paper with a drop of stannous chloride, which gives a pink color with annatto.

After pouring off the ether examine the milk curd for caramel or coal-tar color. If the curd is left white, neither of these colors is present. If caramel has been used, the curd will be of a pinkish-brown color; if the color is due to the coal-tar dye, the curd will have a yellow or orange tint. If now some concentrated hydrochloric acid is poured over the curd the color will change immediately to a bright pink with some coal-tar colors.

Notes.—When the milk is curdled by the acid any added color is carried down by the curd. When this is subsequently treated with ether the fat and annatto are dissolved, leaving any caramel or coal-tar color still in the curd. Since the detection of the latter two colors may depend upon recognizing color in the curd,

[1] *J. Am. Chem. Soc.*, **1900**, 207.

this should always be compared with the curd prepared in the same manner from a sample of milk known to be free from color.

The ordinary tests for caramel as used to show its presence in distilled liquors or vanilla extract are not sufficiently delicate to detect the extremely small quantity which suffices to impart the desired shade of color to the milk. The color imparted to the curd, however, is characteristic and readily recognized.

It is possible that coal-tar dyes may be used which do not give the pink reaction with hydrochloric acid, since this is characteristic in general only of the azo class of dyes. Even in these cases, however, the orange color of the dye is readily perceptible in the separated curd.

Milk colored with an azo dye may occasionally fail to show its presence if the sample is old or somewhat decomposed before being tested. This has been shown by Blyth[1] to be due to the reduction of the dye by nascent hydrogen produced by the growth of certain anaerobic organisms.

Heated Milk.—It is sometimes necessary to determine whether a sample of milk has been heated, as in the process of pasteurization. The statutes in some states require that milk which has been pasteurized shall be labelled "Pasteurized Milk" or "Heated Milk" to distinguish it from raw milk. The presence in raw milk of an enzyme, capable of bringing about color reactions between peroxides and various organic substances, but which is destroyed by heating, has been commonly utilized for this test. Such a reaction is given as a test for hydrogen peroxide, on page 125, and the same test can be used to show heating of the milk, the blue color being given by raw milk but not when the milk has been heated above 75°C.

Advantage may also be taken of the decolorizing power of certain bacteria in raw milk upon the dye, Methylene Blue, reducing it to the colorless leuco-Methylene Blue. Milk which has been heated, on account of the smaller number of bacteria present, exhibits this power to a less degree.

The method[2] is as follows: Mix 20 cc. of milk in a test-tube with 1 cc. of a solution containing 5 cc. of a saturated alcoholic solution of Methylene Blue, 5 cc. of 40 per cent. formaldehyde

[1] *Analyst*, **1902**, 146.

[2] Schardinger: *Z. Nahr. Genussm.*, **1902**, 1113.

(formalin) and 190 cc. of water. Cover the contents of the tube with a layer of liquid petroleum to prevent access of air, and place the tube in a water-bath at a temperature of 45°C. Raw milk will decolorize the reagent in less than 20 minutes. Pasteurized milk will take longer than 20 minutes.

Notes.—Nurenberg and Lythgoe[1] have made a critical study of the above method as compared with the reactions commonly recommended, involving hydrogen peroxide and *p*-phenylenediamine or benzidine, and find it distinctly better. Milk pasteurized at 63°C. and held there for 35 minutes, or at 65°C. for 30 minutes, or at 70°C. and held there for more than 10 minutes, or milk pasteurized at temperatures above 70°C., can be detected by the reaction with Methylene Blue. Milk pasteurized at 75°C. or above can be detected by the use of hydrogen peroxide in conjunction with *p*-phenylenediamine or benzidine, but these reagents do not show it below that temperature and hence are of little practical value.

None of the usual methods will distinguish commercially pasteurized milk 3 days after pasteurization and only the reaction with Methylene Blue will detect it up to and including the second day.

The Schardinger test, using the reagent free from formaldehyde (5 cc. of saturated alcoholic solution of Methylene Blue to 195 cc. of water) may also be used to detect old milk by the rapidity of decolorization. Milk which decolorizes the reagent within an hour is in general too old for consumption. The test, as before, is undoubtedly due to the greater number of bacteria in this older milk.

Interpretation of Results

Apart from the addition of foreign ingredients, such as colors and preservatives, which are detected by the specific tests previously described, the most common forms of adulteration are the addition of water and the removal of cream. By reference to the table on page 107, it will be seen that on account of the variation in the composition of unadulterated cows' milk the detection in all cases is not an easy problem. The variation in the fat content,

[1] *Bur. of Chem., Bull.* **162,** p. 167.

especially, makes it more difficult to show with certainty the partial removal of cream than the addition of water.

This is well shown in the following table in which "A" is a normal milk, "B" the same milk in which the fat has been reduced to 3.6 per cent. by adding water and "C" the same milk in which the fat has been reduced to 3.6 per cent. by skimming.

	A	*B*	*C*
Total solids	12.78	11.34	12.09
Fat	4.00	3.60	3.60
Proteins	2.89	2.60	2.91
Sugar	5.00	4.50	4.98
Ash	0.71	0.64	0.72
Solids not fat	8.78	7.74	8.61

It is seen that in sample *C* it is only the fat that has been decreased to any degree. In fact there is nothing in the figures given for *C* to indicate in any way that the sample is not genuine milk, while in *B* the solids not fat is so low as to be distinctly suspicious of watering.

Composition of Milk of Known Purity.—The average composition of milk, together with the usual and the extreme limits of variation, has already been stated on page 107. The greater number of published analyses of genuine cows' milk have been limited to determination of solids, fat and specific gravity. A more detailed study, including the constants of the copper serum, will be found in Table XIII,[1] which includes the analyses of 33 samples of known purity milk from individual cows, and 4 samples of herd milk, arranged in the order of their percentage of total solids. More extended data, collected by the same observer, has been published elsewhere,[2] but in a form less available for insertion here.

In collecting the samples milk was taken from the heaviest milkers, so as to include a larger proportion of low-grade milk for minimum values. None of the milk could be called exceptionally high grade, as samples were not collected from Jersey or Guernsey cows.

[1] Lythgoe: *Bull. Mass. Bd. Health*, **1910**, 422.
[2] *J. Ind. Eng. Chem.*, **1914**, 899.

Breed	Time since calving (mos.)	Weight of milk (lbs.)	Specific gravity 15°	Total solids (per cent.)	Fat (per cent.)	Proteins (per cent.)	Ash (per cent.)	Solids not fat (per cent.)	Sugar (per cent.)	Copper serum				Natural sour serum		
										Refraction 20°	Specific gravity, 20°/4°	Solids (per cent.)	Sugar (per cent.)	Refraction 20°	Specific gravity, 20°/4°	Ash (per cent.)
Grade Durham	2	15	1.035	14.58	5.10	3.35	0.81	9.48	5.00	39.7	1.0280	6.28	4.43	44.4	1.0292	0.768
Holstein	2	15	1.034	13.65	4.50	3.33	0.72	9.15	4.95	38.4	1.0271	6.05	4.54	43.0	1.0273	0.776
Grade Durham	2	16	1.036	13.52	4.00	3.54	0.72	9.52	5.20	38.9	1.0272	6.09	4.38	43.0	.....	.....
Grade	1	20	1.033	13.36	4.30	3.25	0.81	9.06	5.00	38.3	1.0270	5.96	4.63	42.0	1.0279	0.816
Holstein	4	18	1.034	13.30	4.00	3.54	0.76	9.30	5.00	38.7	1.0269	6.07	4.56	43.1	1.0280	0.830
Grade Durham	5	10	1.032	13.29	4.40	3.14	0.70	8.89	4.70	38.8	1.0275	6.10	4.34	42.2	1.0265	0.784
Grade Ayrshire	2	15	1.034	13.27	4.00	3.36	0.78	9.00	5.40	39.2	1.0282	6.20	4.50	43.3	.....	.....
Grade Swiss	1	12	1.033	13.26	4.20	2.84	0.66	9.06	5.50	39.7	1.0281	6.39	4.78	43.9	1.0286	0.750
Grade Durham	2	16	1.033	13.20	4.35	3.29	0.76	8.85	4.75	38.6	1.0261	5.86	4.48	40.8	1.0259	0.788
Grade Durham	2	15	1.033	13.06	3.80	3.37	0.69	9.26	5.75	39.6	1.0271	6.05	4.82	42.4	1.0274	0.750
Ayrshire	2	16	1.034	13.02	4.10	3.01	0.80	8.92	5.30	30.3	1.0277	6.19	4.47	43.1	1.0286	0.769
Grade Ayrshire	9	10	1.031	12.91	3.90	3.51	0.76	9.01	4.35	37.3	1.0259	5.73	3.94	41.0	1.0254	0.846
Grade Holstein	2	21	1.031	12.85	4.30	3.15	0.68	8.55	5.20	37.1	1.0259	5.66	4.40	39.9	1.0254	0.760
Grade Holstein	2	17	1.032	12.78	4.00	2.89	0.71	8.78	5.05	38.0	1.0274	6.07	4.40	42.1	1.0272	0.768
Holstein	2	18	1.032	12.78	4.00	2.89	0.69	8.78	4.90	38.5	1.0271	6.05	4.40	42.2	1.0269	0.772
Grade Holstein	6	10	1.031	12.66	3.90	2.97	0.76	8.76	4.35	37.6	1.0268	5.85	4.21	41.8	1.0266	0.820
Grade Durham	2	20	1.032	12.64	3.80	3.01	0.62	8.84	5.15	38.7	1.0273	6.11	4.71	41.9	1.0270	0.744
Grade Durham	2	18	1.035	12.58	3.70	3.12	0.74	8.88	5.00	38.4	1.0269	5.93	.....	41.9	1.0271	0.741
Grade Durham	2	18	1.034	12.54	3.50	2.97	0.68	9.04	5.25	38.8	1.0274	6.06	4.14	42.7	.....	.....
Holstein	2	15	1.033	12.50	3.30	3.08	0.74	9.00	5.05	38.6	1.0271	6.03	4.14	42.7	.....	.....
Holstein	4	16	1.031	12.29	3.80	2.65	0.72	8.49	4.85	37.7	1.0266	5.87	4.38	41.8	1.0264	0.755
Grade Holstein	1	10	1.033	12.27	3.40	3.27	0.84	8.87	4.60	37.6	1.0264	5.82	4.11	41.2	1.0259	0.868
Grade Ayrshire	8	18	1.032	12.12	3.50	3.32	0.74	8.62	4.40	37.5	1.0258	5.75	4.25	41.4	1.0262	0.780
Grade Holstein	3	16	1.032	12.08	3.70	2.80	0.78	8.38	4.65	37.0	1.0259	5.70	4.33	40.6	1.0249	0.804
Grade Holstein	4	20	1.032	12.03	3.45	2.89	0.72	8.58	5.00	38.6	1.0273	6.09	4.43	42.1	1.0270	0.800
Grade Holstein	1	20	1.034	12.00	3.10	2.99	0.71	8.90	5.05	38.4	1.0271	6.07	4.61	41.5	1.0270	0.780
Holstein	4	16	1.030	11.77	3.70	2.67	0.71	8.07	4.50	37.2	1.0261	5.79	4.30	39.7	1.0248	0.740
Holstein	½	18	1.033	11.40	3.20	2.48	0.72	8.20	5.00	37.5	1.0261	5.75	4.22	41.0	1.0258	0.800
Holstein	7	15	1.031	11.27	3.15	3.09	0.78	8.12	4.30	36.6	1.0253	5.47	3.73	39.7	1.0244	0.804
Holstein	10	18	1.031	11.21	3.35	2.81	0.75	7.86	4.30	36.3	1.0250	5.37	3.88	38.7	1.0239	0.792
Grade Holstein	3	20	1.031	11.48	3.40	2.78	0.80	8.08	4.50	36.8	1.0256	5.53	4.02	40.6	1.0255	0.800
Holstein	4	16	1.030	10.66	2.85	2.66	0.65	7.81	4.40	36.4	1.0254	5.51	4.22	39.1	1.0242	0.740
	1	28	1.030	10.20	2.65	2.40	0.65	7.55	4.50	36.5	1.0259	5.45	3.76	38.6	1.0234	0.735
Mixed milk[1]	.....	.....	1.033	13.40	4.20	3.13	0.70	9.20	5.10	38.5	1.0260	6.05	4.09	42.2	1.0272	0.780
Mixed milk[2]	.....	.....	1.033	13.08	4.10	3.23	0.76	8.98	4.65	37.7	1.0262	5.84	4.34	41.3	1.0259	0.788
Mixed milk[3]	.....	.....	1.033	12.73	3.80	3.33	0.71	8.93	4.70	38.0	1.0261	5.94	4.22	41.4	1.0272	0.768
Mixed milk[4]	.....	.....	1.032	12.53	3.70	3.35	0.73	8.83	4.75	37.7	1.0259	5.77	4.28	41.5	1.0261	0.808

[1] Grade Ayrshire, Durham, Shorthorn and Holstein cows.
[2] Grade Jersey, Ayrshire and Holstein cows.
[3] Grade Holstein and Grade Jersey cows.
[4] Holstein, Grade Holstein and Grade Jersey cows.

Inspection of this table shows, as would be expected, a great variation in the percentage of fat in the individual samples, the highest being almost 100 per cent. higher than the minimum values. The solids not fat are seen to present a much less variation, and as Lythgoe has pointed out, this variation is due very largely to the changes in protein content, the milk sugar and ash remaining fairly constant. Upon this fact depends the special value of the milk serum in showing adulteration.

Standard Milk.—Nearly all of the states have fixed definite standards for milk which may be sold legally, the standard being based usually upon either the fat content or the total solids. A list of these, from the most recent data available, will be found in Table XIV.

The U. S. Standard[1] is: "*Standard milk* is the fresh, clean, lacteal secretion obtained by the complete milking of one or more perfectly healthy cows, properly fed and kept, excluding that obtained within 15 days before and 10 days after calving, and contains not less than 8.5 per cent. of solids not fat, nor less than 3.25 per cent. of milk fat."

In some cases all that may be necessary is to show by the analysis that the milk does not conform to the legal standard. In certain of the states, however, a legal distinction is made between milk which is simply below standard and milk which has been actually adulterated by skimming or watering. It is therefore of importance to show by the analysis whether water has been added to the milk directly and not through the breed or feed of the cow.

Detection of Watered Milk.—Since in general the water that has been added is no different from the water already present in the milk, it is evident that this form of adulteration can be detected only by showing chemical or physical changes in the milk that could be ascribed only to the addition of water. Methods have been proposed, it is true, based on differences in the added water, such as an abnormally high amount of nitrates, which might have been derived from the polluted barnyard well, but these methods are of little importance.

(*a*) *Solids not Fat.*—Since the variation in proportion of solids not fat in normal milk is much less than the range of total

[1] U. S. Dept. Agr., Office of the Secretary, *Circ.* 19.

TABLE XIV.—LEGAL STANDARDS FOR MILK, 1911[1]

State	Total solids, per cent.	Solids not fat, per cent.	Fat, per cent.
Federal Standard	.....	8.5	3.25
California	11.5	8.5	3.0
Colorado	.....	.....	3.0
Connecticut	11.75	8.5	3.25
District of Columbia	12.5	9.0	3.5
Florida	11.75	8.5	3.25
Georgia	11.75	8.5	3.25
Idaho	11.2	8.0	3.2
Illinois	11.5	8.5	3.0
Indiana	.....	8.5	3.25
Iowa	12.0	.....	3.0
Kansas	11.75	8.5	3.25
Kentucky	12.5	8.5	3.25
Louisiana	.....	8.5	3.5
Maine	11.75	8.5	3.25
Maryland	12.5	.....	3.5
Massachusetts	12.15	.....	3.35
Michigan	12.5	.....	3.0
Minnesota	13.0	9.75	3.25
Missouri	12.0	8.75	3.25
Montana	11.75	8.5	3.25
Nebraska	.....	.....	3.0
New Hampshire	12.0	.....	.....
New Jersey	11.5	.....	3.0
New York	11.5	.....	3.0
Nevada	11.75	8.5	3.25
North Carolina	11.75	8.5	3.25
North Dakota	12.0	9.0	3.0
Ohio	12.0	.....	3.0
Oklahoma	12.51	9.5	3.0
Oregon	.....	9.0	3.2
Pennsylvania	12.0	.....	3.25
Porto Rico	12.0	9.0	3.0
Rhode Island	12.0	.....	2.5
South Dakota	.....	8.5	3.25
Tennessee	.....	8.5	3.25
Texas	.....	8.5	3.25
Utah	12.0	9.0	3.2
Vermont	12.5	9.25	.....
May and June	12.0	.....	.....
Virginia	11.75	8.5	3.25
Washington	12.0	8.75	3.25
Wisconsin	.....	8.5	3.0
Wyoming	.....	8.5	3.25

solids this is of distinct value in showing added water. Although as indicated in the table of limiting values on page 107, the value for solids not fat may go as low as 7.5 per cent., this is rather uncommon, and a fairer minimum would be 7.7 per cent. A value below 7.7 per cent. would certainly be suspicious of added water and if accompanied by correspondingly low values for the constants of the serum could be regarded as direct evidence of adulteration.

(*b*) *Milk Sugar.*—As suggested by Lythgoe,[1] the milk sugar may be employed to even greater advantage than the solids not fat in showing adulteration. Knowing the percentage of solids and of fat the proteins may be calculated by the formulæ given on page 120. Then if 0.7 be assumed as the value for the ash, the milk sugar may be determined by subtracting from the total solids the sum of the other constituents. The expression for the milk sugar would then become

(1) Milk sugar $= T.S. - (F + [0.4(F - 3) + 2.8] + 0.7)$.

(2) Milk sugar $= T.S. - (F + \left[T.S. - \frac{T.S.}{1.34}\right] + 0.7)$.

The portion of the formula enclosed in brackets is the calculated protein in each case. In the case of pure milk the formulæ for calculating the protein will give very similar results, but with adulterated milk they will be divergent, the difference increasing with the extent of adulteration. In the case of watered milk the calculated milk sugar will be too low, ordinarily falling below 4.2 per cent., while as will be shown later, with skimmed milk the milk sugar will be too high, generally above 4.8 per cent.

By means of the two formulæ given above the following table has been calculated, in which are given, for percentages of total solids from 10.5 to 14.0 the corresponding values of fat which would cause the calculated milk sugar to lie between 4.2 and 4.8 per cent. the values assumed for pure milk. If the fat is higher than that stated in the table the milk may be suspected of having added water; if lower, it may be skimmed, the difference increasing with the adulteration. It is true, of course, that a sample which had been both skimmed and watered in the right proportion would fall in the class of good milk. Such a sample, however, would hardly escape detection from its appearance alone.

[1] *Loc. cit.*

TABLE XV.—CALCULATED LIMITING VALUES FOR UNADULTERATED MILK

Total solids, per cent.	Fat, per cent.		Solids not fat, per cent.		Total solids, per cent.	Fat, per cent.		Solids not fat, per cent.	
	Minimum, per cent.	Maximum, per cent.	Minimum, per cent.	Maximum, per cent.		Minimum, per cent.	Maximum, per cent.	Min.-mum, per cent.	Maximum, per cent.
10.5	2.5	2.7	7.8	8.0	12.3	3.7	4.0	8.3	8.6
10.6	2.5	2.8	7.8	8.1	12.4	3.7	4.1	8.3	8.7
10.7	2.6	2.9	7.8	8.1	12.5	3.8	4.1	8.4	8.7
10.8	2.6	3.0	7.8	8.2	12.6	3.9	4.2	8.4	8.7
10.9	2.7	3.0	7.9	8.2	12.7	3.9	4.2	8.5	8.8
11.0	2.8	3.1	7.9	8.2	12.8	4.0	4.3	8.5	8.8
11.1	2.8	3.2	7.9	8.3	12.9	4.1	4.4	8.5	8.8
11.2	2.9	3.2	8.0	8.3	13.0	4.1	4.5	8.5	8.9
11.3	3.0	3.3	8.0	8.3	13.1	4.2	4.5	8.6	8.9
11.4	3.1	3.4	8.0	8.3	13.2	4.2	4.6	8.6	9.0
11.5	3.1	3.5	8.0	8.4	13.3	4.3	4.6	8.7	9.0
11.6	3.2	3.5	8.1	8.4	13.4	4.3	4.7	8.7	9.1
11.7	3.3	3.6	8.1	8.4	13.5	4.4	4.8	8.7	9.1
11.8	3.3	3.7	8.1	8.5	13.6	4.5	4.8	8.8	9.1
11.9	3.4	3.7	8.2	8.5	13.7	4.5	4.9	8.8	9.2
12.0	3.5	3.8	8 2	8.5	13.8	4.6	5.0	8.8	9.2
12.1	3.5	3.9	8.2	8.6	13.9	4.7	5.0	8.9	9.2
12.2	3.6	3.9	8.3	8.6	14.0	4.8	5.1	8.9	9.2

The value of the calculated sugar content for indicating adulteration has been shown graphically by Lythgoe in Table XVI. The upper values for sugar have been calculated by Formula (1), the lower values by Formula (2). The samples falling above the heavy lines may be suspected of being skimmed, those falling below the lower line of being watered, and those falling between the lines are probably unadulterated.

This table, as is also the case with the preceding, should be used only as a preliminary test to select those samples which need to be examined further and to point out the probable character of the adulteration.

(*c*) *Milk Serum.*—If the preliminary calculation, or examination of the table, indicates a possibility of the samples being watered, an examination of the serum should be made. This may be done preferably by the copper sulphate method, which is

TABLE XVI.—CALCULATED LIMITING VALUES FOR MILK SUGAR

| Fat. % | Total Solids | | |

described and the minimum values for pure milk stated on page 122. The following table, due also to Lythgoe, shows the effect of systematic watering on the composition of the milk and the constants of the serum in the case of a milk which was above the average in solids not fat and refraction.

TABLE XVII.—COMPOSITION OF A SAMPLE OF MILK SYSTEMATICALLY WATERED

Added water, per cent.	Solids, per cent.	Fat, per cent.	Solids not fat, per cent.	Copper serum		
				Refraction, 20°	Specific gravity, 20°/4°	Solids, per cent.
0	13.18	4.20	8.98	38.5	1.0272	6.09
10	11.86	3.78	8.08	36.4	1.0249	5.57
20	10.54	3.36	7.18	34.4	1.0233	5.05
30	9.23	2.94	6.29	32.4	1.0211	4.56
40	7.91	2.52	5.39	30.6	1.0194	4.10
50	6.59	2.10	4.49	28.6	1.0174	

It is seen that each 5 per cent. of added water lowers the refraction by one scale division, hence with average milk, refracting below 38°, 10 per cent. of added water could be detected and with rich milk 15 per cent. can usually be found.

In the particular case given in the table it is evident that the addition of 10 per cent. of water would escape detection.

Detection of Skimmed Milk.—Watering milk does not in general change the relation of the various constituents to one another, since these are all reduced in the same proportion, but removing the fat does change these ratios. It is immaterial whether the milk is skimmed by the actual removal of some of the fat or whether separator skim milk is added to normal milk. In either case the resulting product will have its fat content largely reduced while the proteins and sugar suffer but little change. In normal milk, especially in the mixed milk of a herd, the percentage of fat is rarely less than the protein (see table, page 131). In 5500 analyses of American milks compiled by Van Slyke, with a fat content between 3 and 5 per cent. the average amount of fat was 3.92 per cent. and the average amount of proteins 3.20 per cent. If

such milk be skimmed the fat may be reduced to 1 per cent. or even to 0.1 per cent. but the protein content will still be approximately the same as before. In general, it may be said that a protein-fat ratio greater than 1.0 indicates skimming, the amount being greatest in samples showing the highest ratio. In the calculation of milk sugar by the formulæ given on page 120, the same effect will be noticed, that is, the skimming will lower the fat or the solids to a greater extent than the protein. Hence the proteins calculated from the fat or total solids will be too low and the calculated milk sugar will be too high. For practical purposes the limit for unskimmed milk may be set at 4.8 per cent., values above this being suspicious of skimmed milk.

In addition to this preliminary test the milk may be with certainty declared skimmed if the fat falls below 2.2 per cent. (the minimum value given in the table on page 107), the solids not fat remaining above the average value of 8.5 per cent. If the fat is above 2.2 per cent and below 3.5 per cent. the presence of skimmed milk may be confirmed by making a Kjeldahl nitrogen determination on the suspected sample and calculating the proteins by the factor 6.38. If the proteins exceed the fat, as stated in the preceding paragraph, the sample is skimmed. If, however, the fat is above 3.5 per cent. this procedure will no longer suffice, since the proteins rarely exceed 3.5 per cent. In these few cases the skimming can be judged only from the high specific gravity, high solids not fat and correspondingly low fat.

Specific Gravity of Milk Solids.—The specific gravity of the milk solids is sometimes used to show skimming. Fleischmann's formula for calculating this is

$$x = \frac{TS}{TS - \dfrac{(100 \times Gr) - 100}{Gr}}$$

when TS = the total solids and Gr the specific gravity of the milk.

Example.—A sample of milk contains 12.85 per cent. of milk solids and has a specific gravity of 1.031. Required the specific gravity of the milk solids.

$$x = \frac{12.85}{12.85 - \dfrac{(100 \times 1.031) - 100}{1.031}} = \frac{12.85}{12.85 - 3.006} = 1.306$$

The specific gravity of the solids of normal milk varies between 1.25 and 1.34. It is not changed by watering the milk but is increased by removing the fat or adding skimmed milk. A value above 1.32 is suspicious while a specific gravity of the milk solids above 1.40 is regarded as conclusive evidence of skimming.

Cream

The term *cream* in modern dairy practice is almost invariably applied to the product prepared by centrifugal separators, a method which causes a much more nearly complete separation of the fat from the milk than was possible with the older methods.

Composition.—The following figures illustrate the composition of the cream and skim milk obtained by centrifugal separation of a rather rich sample of milk:

Table XVIII.—Composition of Milk, Cream and Skim Milk

	Milk, per cent.	Skim milk, per cent.	Cream, per cent.
Fat	5.05	0.20	21.95
Total solids	14.10	9.6	26.98
Lactose	4.70	5.05	3.32
Casein	3.50	3.62	2.02
Ash	0.79	0.78	0.58
Specific gravity	1.032	1.034	1.015

The *Federal standard* for cream requires a fat content of not less than 18 per cent.

Cream has the same constituents that are found in milk, practically the only difference between the two being in the greatly increased fat content and the consequent decrease in the amounts of the other constituents. Typical analyses of both "light" and "heavy" cream are given below:

	Total solids, per cent.	Fat, per cent.	Casein, per cent.	Sugar, per cent.	Ash, per cent.
Heavy cream	52.77	49.19	.....	.....	0.26
" "	28.98	21.95	3.02	3.32	0.58
Light cream	21.07	13.88	2.76	3.75	0.68

Forms of Adulteration.—Since cream is valued for its content of fat, adulteration may consist either in a deficiency of fat or in the substitution of foreign fat for the whole or a part of the butter fat.

The latter form of adulteration is carried out in recent times by means of the "homogenizer," a machine by which the melted fat is introduced into milk or skim milk in such small particles and so intimately mixed that a nearly perfect emulsification results and the product has the appearance of genuine cream. Cream is at times made in this way from butter which has been thoroughly washed and added to skim milk, the advantage of the process being that the fat can be stored more economically in the form of butter until the period of greatest demand. Considerable use is found for the homogenized product in the manufacture of ice cream, for which there is an extra demand in hot weather or at holiday seasons. Cocoanut oil has likewise been reported as being used in cream, as well as oleomargarine.

Substances are also added to cream in order to thicken it, the thickness of the cream being the popular criterion of its richness in fat. If the cream or the milk itself prior to passage through the separator has been pasteurized, the product will in many cases be thinner than corresponds to the actual amount of fat present, which is another reason for the use of artificial thickeners. Substances added for this purpose comprise *gelatin*, *agar-agar*, a substance resembling gelatin in its properties and obtained from a Japanese seaweed, and *sucrate of lime*.

The use of unsweetened condensed milk for thickening cream has also been reported.[1] (See also page 143.)

Preservatives constitute another form of adulteration, those employed being the same as under Milk.

Methods of Analysis

Fat.—The fat may be determined by the Babcock method as described on page 113, but on account of the thickness of the cream and its high fat content, certain modifications of the method are advisable. Test bottles of greater capacity and different gradua-

[1] Lythgoe: *Ann. Rept. Mass. Bd. Health*, **1911**, p. 430.

tion, sold as "cream bottles," should be used, and the sample of cream taken should be weighed rather than measured. If many determinations are to be made the special "cream balances" sold by dealers in chemical supplies will be found convenient.

Directions.—Weigh about 10 grams of the mixed sample into the "cream bottle," add 5 or 6 cc. of water and proceed as described on page 113. Since the bottles are calibrated for a weight of 18 grams, the reading should be corrected by the following formula:

$$F = \frac{18R}{W}$$

where F = per cent. of fat; R = the reading; and W = the weight of cream taken.

Note.—If the form of cream bottle with wide neck is used, the meniscus of the fat column will be so considerable as to produce an error in the reading. According to Babcock and Farrington[1] this may be overcome by adding a few drops of alcohol previously saturated with fat.[2]

Gottlieb Method.—Weigh out 0.5 to 1.0 gram of the cream and carry out the determination as under Milk, page 114.

Total Solids.—Weigh 2 grams into a tared, flat-bottomed platinum dish, add about 5 cc. of water and finish the determination as on page 111.

Lactose, proteins and **ash** are determined as previously described under Milk, pages 112 to 119, using preferably aliquot portions of a weighed sample diluted with water to definite volume, such as 25 grams diluted to 100 cc.

Foreign fats may be detected by separating a considerable quantity of the fat in a manner generally similar to that employed in the Gottlieb process and examining the dried fat by the methods described under Butter, pages 200 to 213.

Preservatives.—Use a diluted sample and follow the methods outlined on pages 124 to 126.

Detection of Thickeners.—*Gelatin.*[3]—To about 10 cc. of the cream add about an equal volume of water and mix thoroughly.

[1] *Wis. Expt. Sta., Bull.* **195**; Leach: Food Analysis, 3d Ed., p. 196.

[2] Prepared by shaking warm alcohol with a small quantity of butter until saturated.

[3] Stokes: *Analyst*, **1897**, 320.

Then add 10 cc. of acid mercuric nitrate (use the solution given on page 115, diluted with ten times its volume of water), shake, and after allowing the mixture to stand 5–10 minutes, filter. If a considerable amount of gelatin is present the filtrate will be somewhat turbid. To a part of the filtrate add half its volume of a saturated, aqueous solution of picric acid. A yellow precipitate will be produced if gelatin is present.

Note.—Since the acid mercuric nitrate itself will cause a turbidity with picric acid if present in large excess, a positive test should always be confirmed by repeating the test in exactly the same manner with a sample known to be pure, in order to be certain that the precipitate is not due to the reagents. The test will detect about 1 part of gelatin in 10,000 parts of water but is somewhat less delicate with cream.



FIG. 41. *Arachnoidiscus Ehrenbergii* × 100. The smaller oval diatoms are *Cocconeis*. (LEFFMAN AND BEAM.)

Agar-agar.—Dilute a considerable quantity of cream (50 cc.) with twice its volume of water, add 5 cc. of 10 per cent. calcium chloride and heat in the water-bath until the precipitate settles. Filter clear while still hot, cool the filtrate and add two-thirds its volume of 95 per cent. alcohol. Filter off the precipitated agar and boil it a few minutes with a small quantity of water, filter hot and evaporate the filtrate on the water-bath to 5 cc. If an appreciable amount of agar is present the solution will gelatinize on cooling.

The presence of agar may often be confirmed by transferring the jelly or the concentrated solution obtained in the test just described to a Kjeldahl flask (see page 26) and destroying the organic matter by heating with sulphuric and nitric acids. The material should be heated with strong sulphuric acid until fumes are given off and then several portions of strong nitric acid (sp.

gr. 1.42) added at intervals. When the solution is nearly colorless dilute and wash several times with water by decantation, or preferably by using a centrifuge. Transfer a drop of the sediment to a microscope slide, cover with a cover-glass and examine under the microscope. Agar frequently, although not always, contains numerous diatoms, of which the more common are species of *Arachnoidiscus* and *Cocconeis* (Fig. 41).

Homogenized Cream.—This can usually be recognized by examination with the microscope, the effect of the process being to render the fat globules much more uniform in size as well as smaller than in raw cream. Waters[1] found as a result of 100 measurements of raw cream that the average diameter of the fat globules was 2.44μ. The same number of measurements carried out on a homogenized sample gave an average diameter of 0.66μ. A characteristic difference also noticed was the greater uniformity in size of the globules of homogenized cream. The globules of the raw cream varied in size by as much as 6 or 8μ, while in the homogenized cream they rarely differed in size more than 1μ. The tests were made by adding 1 cc. of cream to 99 cc. of water, shaking vigorously 3 minutes, diluting and shaking again in the same way twice more, giving a final dilution of 1:100,000. The globules were measured in a "hanging drop" using an oil immersion lens. The shaking doubtless affects the globules somewhat but is necessary to obtain the proper dilution.

Condensed Milk.—The use of the sweetened product in any quantity would be shown by the pronounced test for sucrose (see below). The presence of the unsweetened evaporated milk would be indicated by the increased proportion of non-fatty solids and the abnormally high refraction of the serum. This is shown by the following analysis, in which the first sample is a pure cream and the others are adulterated in this manner.

	Fat, per cent.	Protein, per cent.	Lactose, per cent.	Ash, per cent.	CaO, per cent.	Refraction of copper serum
(*a*)	38.8	2.20	2.80	0.48	0.106	37.5
(*b*)	27.8	3.08	3.96	0.66	0.166	42.9
(*c*)	37.4	3.05	3.94	0.66	0.152	.....

[1] Thesis, Mass. Inst. Technology, 1910.

Sucrate of Lime.—The presence of this may be shown by qualitative tests for sucrose, or somewhat less satisfactorily, by the increase in the calcium content of the cream.

(*a*) *Baier and Neumann's*[1] *test for sucrose* has been thoroughly studied by Lythgoe[2] who gives the following directions:

To 25 cc. of cream add 10 cc. of 5 per cent. uranium acetate solution, shake well, allow to stand for 5 minutes and filter. To 10 cc. of the clear filtrate (the entire filtrate if less than 10 cc.) add a mixture of 2 cc. saturated ammonium molybdate solution and 8 cc. dilute hydrochloric acid (1 volume of acid of 1.12 sp. gr. to 7 volumes of water), and place in a water-bath at 80°C. for 5 minutes. If sucrose is present the solution will be of a prussian blue color, which should be compared with the standard prussian blue solution. This is prepared by adding a few drops of potassium ferrocyanide and 5 drops of 10 per cent. hydrochloric acid to 20 cc. of water containing 1 cc. of a 0.1 per cent. ferric chloride solution.

Notes.—The test is very similar to that proposed by Cotton (page 123) except that it is made on the serum. As in the other test the color is due to reduction of the molybdic acid and is not characteristic of sucrose.

Occasionally a sample of pure milk will be found which will give the test, but to a much less degree than the standard. Moreover the color in this case can be removed by filtration, leaving a green filtrate, while the color due to sugar is not thus removed.

Stannous chloride, ferrous sulphate and hydrogen sulphide cause a similar reduction in the cold, but will not interfere with the test unless present in such quantities as to change the character of the sample. If no blue color appears before heating, these substances are absent.

The resorcinol test, page 123, may also be used on the serum prepared with uranium acetate as described above.

(*b*) *From the Calcium Content.*—Weigh 25 grams of cream into a platinum dish, dry several hours in the water-oven and ignite carefully to ash, preferably in a muffle. Boil the residue in the dish with 20 cc. of dilute sulphuric acid, avoiding loss by spatter-

[1] *Z. Nahr. Genussm.*, **1908,** 51.

[2] *Bur. of Chem., Bull.* **122,** p. 52; **132,** p. 122.

ing, neutralize with dilute sodium hydroxide and finally add a few drops of acetic acid. Filter, add 1 gram of sodium acetate and ammonium oxalate in excess. Boil, filter and wash with hot water. Either determine the calcium oxalate by the ordinary gravimetric method, or dissolve it in hot dilute sulphuric acid and titrate while hot with tenth-normal potassium permanganate. Calculate as CaO.

Note.—The percentage of calcium in cream varies with the amount of fat, the samples containing a higher percentage of fat having less calcium. From the examination of many genuine samples Lythgoe[1] has arranged the following table giving the maximum amount of calcium oxide permissible in genuine cream. Pure cream as on the market will ordinarily fall quite a little below the maximum values of the table, since this is largely made from milk which has been pasteurized, the latter process rendering some of the calcium insoluble and removing it from the cream in the separator.

TABLE XIX.—RELATION BETWEEN FAT AND CALCIUM IN CREAM

Fat, per cent.	Maximum CaO, per cent.	Fat, per cent.	Maximum CaO, per cent.	Fat, per cent.	Maximum CaO, per cent.
15	0.181	28	0.149	41	0.118
16	0.178	29	0.146	42	0.115
17	0.175	30	0.144	43	0.113
18	0.173	31	0.141	44	0.110
19	0.171	32	0.139	45	0.108
20	0.169	33	0.137	46	0.106
21	0.166	34	0.134	47	0.103
22	0.164	35	0.132	48	0.100
23	0.161	36	0.129	49	0.098
24	0.158	37	0.127	50	0.096
25	0.156	38	0.124	.....	.....
26	0.154	39	0.122	.....	.....
27	0.151	40	0.120	.....	.....

Selected References

FARRINGTON AND WOLL.—Testing Milk and its Products.
GROTENFELT.—The Principles of Modern Dairy Practice.

[1] *Loc. cit.*

Leffman.—Milk. In Allen's Commercial Organic Analysis, 4th Edition, Vol. VIII.
Revis and Bolton.—Milk Products. In Allen's Commercial Organic Analysis, 4th Edition, Vol. VIII.
Richmond.—Dairy Chemistry.
Russell.—Dairy Bacteriology.
Van Slyke.—Modern Methods of Testing Milk and Milk Products.

CHAPTER V

EDIBLE FATS AND OILS

Composition.—The fats and oils, excluding from the term *oil* the mineral and essential oils, are mixtures of glyceryl esters of the fatty acids, a particular oil usually containing a number of these esters of various acids. There is no difference in general composition between the fats and the oils, the latter name being given to those fats which are liquid at ordinary temperatures.

General Properties.—When pure, the oils and fats are free from color, odor and taste, their customary appearance in this respect being due to impurities which have not been removed. They are almost completely insoluble in water and, with the exception of castor oil, nearly so in cold alcohol. Hot alcohol dissolves small quantities and practically all of the oils and fats dissolve readily in chloroform, ether, carbon bisulphide, carbon tetrachloride and petroleum ether.

When heated, little change is produced, except in the case of the drying oils, until decomposition occurs beyond 250°C., with the formation of the intensely irritating *acrolein*. When acted on by the oxygen of the air, especially in the presence of light and moisture, free fatty acids are liberated and altered with the accompanying production of a disagreeable odor and acrid taste, the fat or oil then being termed "rancid."

The chief properties of the fatty acids more commonly occurring in edible fats and oils are tabulated below.[1]

The acids of the acetic series are all saturated monobasic acids, of which the first four alone can be distilled at atmospheric pressure without decomposition. By reference to the table it will be observed that as the molecular weight of the acids increases there is a corresponding rise in the melting and boiling points

[1] Leach: Food Analysis, 3d Ed., p. 471; Allen's Com. Org. Anal., 4th Ed., Vol. II, p. 372.

Name	Formula	Melting point C.	Boiling point C.	Sp. gr. 20°C.	Occurs in
		Acetic Series $C_nH_{2n}O_2$			
Butyric	$C_4H_8O_2$	6.5°	162.3°	0.959	Butter
Caproic	$C_6H_{12}O_2$	.....	200.0°	0.924	Butter, cocoanut oil.
Caprylic	$C_8H_{16}O_2$	16.5°	236.0°	0.910	Butter, cocoanut oil.
Capric	$C_{10}H_{20}O_2$	31.3°	269.0°	.....	Butter, cocoanut oil.
Lauric	$C_{12}H_{24}O_2$	43.6°	.....	0.883	Cocoanut oil, palm oil.
Myristic	$C_{14}H_{28}O_2$	53.8°	.....	0.858 (60°)	Cocoanut oil, nutmeg, lard.
Palmitic	$C_{16}H_{32}O_2$	62.6°	.....	0.85 (60°)	Nearly all fats and oils.
Stearic	$C_{18}H_{36}O_2$	69.3°	.....	.....	Most fats.
Arachidic	$C_{20}H_{40}O_2$	77.0°	.....	.....	Peanut oil.
Lignoceric	$C_{24}H_{48}O_2$	80.5°	.....	.....	Peanut oil.
		Oleic Series $C_nH_{2n-2}O_2$			
Hypogæic	$C_{16}H_{30}O_2$	33.0°	.....		Peanut oil.
Oleic	$C_{18}H_{34}O_2$	14.0°	.....	.....	Most fats and oils.
Erucic	$C_{22}H_{42}O_2$	33°-34°	.....	....	Rape and mustard oils.
		Linoleic Series $C_nH_{2n-4}O_2$			
Linoleic	$C_{18}H_{32}O_2$	Below 18°	.....	.....	Drying and semidrying oils.

while the reverse is true of the specific gravity and the solubility of the acids in water and in cold alcohol. In a general way the same is true of their compounds, the glyceryl esters of the acids, which occur in the fats.

In distinction from these, the acids of the oleic series are unsaturated, as seen in the structural formula for oleic acid below. It is therefore possible to add two atoms of halogen to form a saturated compound. The great value of this characteristic from an analytical standpoint is seen in the important determination of the iodine value on page 160. It will be observed from the table that the acids of this series have lower melting points than the corresponding acids of the acetic series, hence they are found more largely in the liquid oils than in the solid fats.

A peculiar characteristic of the acids of the oleic series is that by the action of nitrous acid they are changed to solid isomers.

Thus oleic acid $CH_3 - (CH_2)_7 - CH = CH - (CH_2)_7 - COOH$, with a melting point of 14°, is changed to the isomeric crystalline *elaidic acid*, which melts at 51°. The so-called *elaidin test*, dependent upon this property, will be found described in standard works on oil analysis, but for the general examination of edible oils affords no information that is not given by other and more reliable methods.

Another property of the unsaturated acids which is of analytical importance is the solubility of their lead salts in ether. If a mixture of saturated and unsaturated fatty acids be precipitated with lead acetate and the insoluble lead soaps thus formed be allowed to stand with ether, the lead oleate, hypogeate and linoleate will be dissolved, leaving the salts of the saturated acids. This method is used in the Renard test for peanut oil (page 184) to separate the arachidic acid and facilitate its crystallization.

Linoleic acid, the important constituent of the "drying oils," resembles the unsaturated acids of the previous group, but is capable of absorbing four atoms of halogen and will also absorb oxygen from the air, becoming thick and viscid. When exposed in a thin layer to air it forms a varnish.

Classification.—In any systematic study of the oils it is advantageous to group together those which bear some resemblance in their physical properties or chemical nature. The classification given below, condensed from that given in Allen's Commercial Organic Analysis, comprises the important edible oils.

(*a*) *Olive oil group* (olive, almond, peanut), *vegetable oleins* containing chiefly olein with smaller amounts of the glycerides of palmitic, stearic, arachidic and in some cases linoleic acids. They are characterized by rather low iodine and saponification numbers.

(*b*) *Rape oil group* (rape, mustard-seed oils), fatty oils from seeds of the *Cruciferæ* having distinctly lower saponification values than the oils of the preceding group.

(*c*) *Cotton seed oil group* (cotton seed, corn, sesame), *semi-drying* oils consisting chiefly of olein and linolein. They have in general fairly high iodine values.

(*d*) *Linseed oil group* (poppy, sunflower), differing from the semi-drying oils in the greater proportion of the glycerides of the highly unsaturated acids, linoleic and linolenic. The most im-

portant member of the group commercially, linseed oil, need not be included among the edible oils.

(*e*) *Cocoa-butter group* (cocoa butter, cotton seed stearin), *vegetable fats* comprising chiefly the glycerides of the higher fatty acids as myristic, palmitic, stearic and oleic. They contain only small amounts of the glycerides of acids below myristic.

(*f*) *Cocoanut oil group* (cocoanut oil, palm nut oil), *vegetable fats* distinguished from the preceding group by higher saponification values (showing glycerides of lower fatty acids) and low iodine values (showing the small proportion of unsaturated acids). The larger proportion of lower fatty acids is shown also by their relatively high Reichert-Meissl numbers. (See page 175.)

(*g*) *Lard oil group* (lard oil), *animal oleins*, liquid at ordinary temperatures and consisting mainly of olein. They correspond to the vegetable oils of group (*a*) although having lower iodine values.

(*h*) *Tallow group* (butter, beef fat, lard, mutton fat, tallow), *animal fats* which in distinction from the previous group are solid at ordinary temperatures. They consist chiefly of olein, palmitin and stearin, with the notable exception of butter, which contains considerable amounts of the glycerides of butyric and other lower fatty acids.

Methods of Analysis

Object of Oil Analysis.—The term "oil analysis" is somewhat of a misnomer in that as ordinarily carried out it is entirely different from an analysis in which the percentage of the constituents present is determined. The object is usually to determine the purity of a given sample or in the case of a product of unknown source to determine what oil or oils are present. It is therefore customary to determine the "analytical constants" of the oil or mixture, by which is meant those chemical or physical tests which give characteristic values for the different oils, the variation in the "constant" being only slight for a particular oil if pure. It will be evident that for this reason testing a known oil for purity will be in general less difficult than determining what oils may be present in an unknown mixture. It is apparent also that the differences in these constants for the various oils must be due en-

tirely to differences in the fatty acids which are present, glycerin being common to all.

Sherman[1] has pointed out concisely that the differences to be expected are due mainly: (1) to differences in the mean molecular weight of the acids that are present, or the relative proportion of acids of high and of low molecular weight; (2) in the relative number of "double bonds," depending upon the proportion of unsaturated acids such as oleic and linoleic, that are present. Of the constants which are described below, typical of the first class would be the Saponification Number, which is a measure of the molecular weight of the acids present, and the Reichert-Meissl Number, which depends upon the proportion of acids of low molecular weight. Of those which depend upon the proportion of unsaturated acids present, the Iodine Value and Maumené Number are typical.

Certain of the constants, as the Specific Gravity and Melting Point, are more general, being simply composites of the values due to all the acids present.

Physical Methods

Specific Gravity.—The specific gravity of oils may be conveniently determined by the Westphal balance or pyknometer at the standard temperature of 15.5°C. Owing to the high coefficient of expansion of oils the precautions detailed in the chapter on General Methods, pages 1 to 4, should be carefully observed. If it is found preferable to make the determination at room temperature the result should be corrected by the following formula:

$$Sp_{15.5} = k\, Sp_t$$

where Sp_t is the specific gravity obtained at $t°$ and k is a factor varying with the temperature. The values of this factor for ordinary temperatures are:[2]

16°	1.00035	21°	1.00391
17°	1.00106	22°	1.00462
18°	1.00177	23°	1.00534
19°	1.00248	24°	1.00605
20°	1.00319	25°	1.00677

[1] Organic Analysis, 2d Ed., p. 144.

[2] Wright: *J. Soc. Chem. Ind.*, 1907, 513.

If it is desired to obtain the specific gravity at a temperature considerably above room temperature the Sprengel tube (page 2) will be found especially suitable, as it may be easily filled with the oil, then suspended in a beaker of water and adjusted when at the desired temperature. If it is then removed and cooled to room temperature it can be wiped dry and weighed with no danger of loss of contents.

In the case of fats which are solid at ordinary temperature the determination is best made at a temperature considerably above their melting point, conveniently at 40° or 50°C., the Sprengel tube being previously warmed, then filled with the melted fat, the test being finished as usual. The determination may be calculated to 15.5° by the formula

$$Sp_{15.5} = Sp_t + k(t - 15.5)$$

where Sp_t is the value obtained at the temperature t and k is the change in specific gravity for 1°C. The values for k for the common edible fats are:[1]

Cocoa-butter	0.000717
Tallow	0.000673
Lard	0.000650
Butter fat	0.000617
Cocoanut stearin	0.000674
Cocoanut oil	0.000642
Palm oil	0.000657
Stearic acid	0.000750
Oleic acid	0.000656

If only a few drops of oil or a small quantity of fat is available for the determination, the gravity may be obtained by placing the sample in a cylinder containing dilute alcohol.[2] By the cautious addition of alcohol or water the mixture may be so adjusted that the oil globules or particles of fat neither rise nor fall but remain in equilibrium in the liquid, which should be at 15.5°C. The specific gravity of the liquid is taken and is obviously the same as that of the oil.

Note.—The specific gravity is a constant which does not vary greatly for any given kind of oil provided it is examined while

[1] Allen: Com. Org. Anal., Ed. 4, Col. II, p. 49.
[2] Hagar: *Pharm. Zentralh.*, **1879**, 132.

pure and fresh, but is readily affected by age, rancidity and any special treatment of the oil.

The different values obtained with different oils are due to differences in the fatty acids present, an increase in molecular weight being accompanied by a decrease in specific gravity, the opposite effect being produced by greater proportions of unsaturated and hydroxyl acids.

TABLE XX.—COMMON EDIBLE OILS IN THE ORDER OF THEIR SPECIFIC GRAVITIES[1]

Cocoa-butter	0.960	Cotton seed oil	0.922
Beef tallow	0.947	Palm oil	0.922
Mutton tallow	0.944	Cotton seed stearin	0.921
Butter fat	0.936	Peanut oil	0.918
Lard	0.934	Almond oil	0.917
Cocoanut oil	0.926	Mustard oil	0.917
Poppyseed oil	0.925	Olive oil	0.916
Sunflower oil	0.925	Lard oil	0.915
Corn oil	0.924	Rape oil	0.915
Sesame oil	0.923		

Refractive Index.—The manipulation of the Abbe refractometer and the principle on which it is based have been described on page 7, under General Methods. The determination is quickly and easily made, using a few drops of the oil or melted fat. The standard temperature for reporting the refractive index is 25°C. in the case of oils and 40° for the solid fats, and it is desirable to make the readings at nearly these temperatures. If the temperature is slightly above or below the standard the reading should be corrected by 0.000365[2] for each degree difference, remembering that the index of refraction increases as the temperature decreases.

Although the Abbe refractometer is the one best adapted to general laboratory use, in the case of fats and oils somewhat more

[1] Average values at $\frac{15.5°}{15.5°}$; see Table XXIX, p. 175, for usual variations.

[2] Tolman and Munson: *J. Am. Chem. Soc.*, **1902,** 754. According to Richmond (*Analyst,* **1907,** 44) the corrective value is nearer 0.00038. If either value be used, by making the determination at a temperature as near as practicable to the standard and yet have the fat liquid, the error in the final result will be inappreciable.

delicate readings can be obtained with the *butyro-refractometer*, an instrument specially designed for the edible fats and oils and restricted to the range of values which they cover. Directions for the use of this instrument and tables for converting its readings to indices of refraction may be found in Leach's Food Analysis.

Notes.—The great value of the refractive index as an analytical constant lies in the ease with which the test may be applied and the small sample that is needed. For this reason it is often used in the routine examination of numbers of samples as a preliminary or sorting test. With oils in general the refractive index varies as does the specific gravity, both increasing with an increased percentage of unsaturated acids, a notable exception being butter fat, which, on account of the high proportion of acids of low molecular weight, has a lower refractive index than other animal fats, although its specific gravity is higher.

There is also, as various investigators have pointed out,[1] a definite relation between the refractive index and the iodine number. Attempts have even been made, although unsuccessfully, to calculate the latter value from the former.

The refraction of the oils is considerably influenced by heat and age, both tending to increase it.

TABLE XXI.—COMMON EDIBLE OILS IN THE ORDER OF THEIR REFRACTIVE INDICES[2]

Poppyseed oil	1.473	Lard oil	1.467	
Corn oil	1.472	Olive oil	1.467	
Mustard oil	1.472	Lard	1.452	(at 60°C.)
Sunflower oil	1.472	Palm oil	1.451	(at 60°C.)
Rape oil	1.471	Tallow	1.451	(at 60°C.)
Sesame oil	1.471	Cocoa butter	1.450	(at 60°C.)
Cottonseed oil	1.471	Butter fat	1.447	(at 60°C.)
Almond oil	1.469	Cocoanut oil	1.441	(at 60°C.)
Peanut oil	1.468			

Melting Point.—(*a*) *Capillary Tube Method.*—Melt a small quantity of the fat at as low a temperature as possible and draw it up into several thin-walled capillary tubes about 3 cm. long. Place these on ice for not less than 12 hours. Attach one of the

[1] Beckurts and Seiler: *Z. angew. Chem.*, **1895**, 612; Partheil and von Velsen: *Z. Nahr. Genussm.*, **1899**, 794.

[2] Average values at 25°C.; for the usual variations see Table XXIX, page 175.

tubes thus prepared to a delicate thermometer graduated to tenths of a degree, using a small rubber ring clipped from a piece of tubing. The tube should be so attached that the fat is as close as possible to the bulb of the thermometer. The thermometer is supported by a cork or a clamp so that its bulb is immersed in water in a wide test-tube, which in turn rests in the neck of a round-bottomed flask also containing water (Fig. 42). The water is heated gradually at a rate not exceeding 0.5° per minute until the fat melts. The temperature at which the fat becomes transparent is taken as the melting point. This should not be confused with the *softening point*, usually several degrees below the true melting point, at which the fat may gradually change its position in the capillary. The mean of several determinations should be taken as the final value.

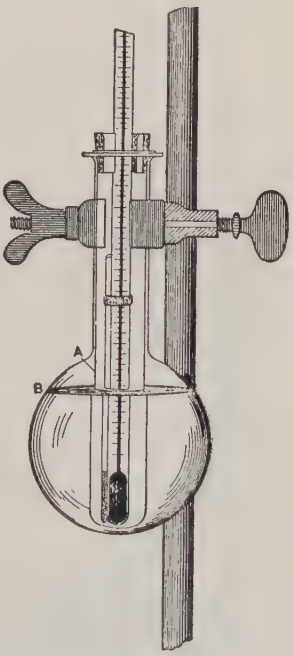


FIG. 42.—Melting-point apparatus. (MULLIKEN.)

(*b*) *Wiley's Method.*[1]—On account of the difficulty in determining the precise point at which the fat becomes liquid, if exact results are desired the following method is preferable. In the case of determinations on the fatty acids themselves, however, these being soluble in alcohol, the capillary tube method must be employed.

1. Prepare discs of the fat by allowing several drops of the melted fat to fall from a height of about 15–20 cm. on to a smooth piece of ice floating in recently boiled distilled water. Thin discs about 1 cm. in diameter will be formed and can be removed by forcing the ice below the water, when the disc will float and can be taken up on a steel spatula or knife blade previously cooled in ice water. The discs should preferably be allowed to stand in the refrigerator several hours before being used to determine the melting point.

[1] Wiley: Agricultural Analysis, Vol. III, p. 324.

2. Half fill a wide test-tube with hot, recently boiled, distilled water, then carefully pour a nearly equal volume of hot, recently boiled alcohol down the side of the tube so as to form a layer above the water. Suspend the test-tube in a wider tube or tall beaker nearly filled with water and provided with a bent glass tube reaching nearly to the bottom through which air can be blown for stirring (Fig. 43). The test-tube containing the alcohol and water is placed in the beaker containing water and a little ice until cold. Drop in a disc of fat, which will sink to a point where the specific gravity of the alcohol-water mixture is the same as its own. Lower a delicate thermometer, reading to tenths of a degree, into the tube until the bulb is at the level of the disc and slowly heat the beaker, keeping the water well stirred. When the disc begins to shrivel, indicating that the temperature is about 5° from the melting point, heat very slowly so that not less than 5 minutes are required for the temperature to rise 2°. Occasionally move the thermometer bulb gently around the disc of fat as the melting point is approached. The reading is taken when the fat becomes a sphere. Having determined the approximate melting point by a preliminary trial, duplicate determination should agree within 0.2°.

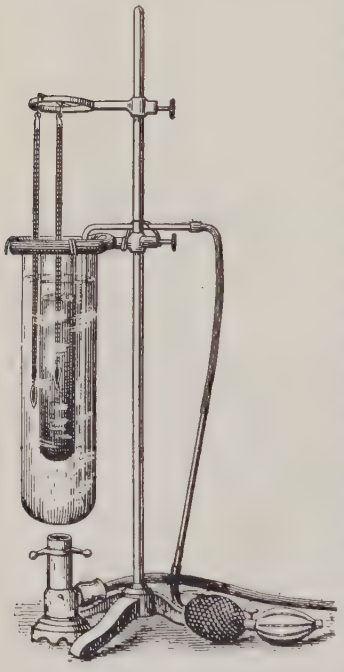


Fig. 43.—Wiley's melting-point apparatus.

It is important to use a very thin disc of fat and to add the alcohol to the water while both are still hot, since otherwise the mixture will contain many air-bubbles which will gather on the disc of fat as the temperature rises and finally force it to the surface.

Notes.—Since the fats are mixtures of various glycerides they

do not show the sharp melting point characteristic of pure organic compounds, but first soften, then shrink in volume and gradually form a transparent liquid. Hence different values may be recorded, depending on whether the beginning of fusion or the transparent point is observed, and to secure concordant results a uniform procedure must be followed.

If the fat consists mainly of glycerides of fatty acids of the saturated or acetic series, its melting point will increase as the mean molecular weight of the mixed acids increases, while if unsaturated acids are present, the melting point decreases proportionally.

Some of the fats have more than one melting point. Thus tallow, if previously melted at a fairly high temperature, shows a melting point of 36°. If the previous melting, however, be carefully made at 36°–38°, and the melting point of the cooled fat be taken again it will be nearer 47°. This has been shown[1] to be due to the presence of two isomeric forms of certain of the glycerides, one of lower melting point gradually changing into the other of higher melting point.

TABLE XXII.—COMMON EDIBLE FATS IN ORDER OF THEIR MELTING POINTS[2]

Tallow	45°C.
Lard	40°C.
Palm oil	40°C.
Cotton seed stearin	33°C.
Butter fat	32°C.
Cocoa butter	30°C.
Cocoanut oil	25°C.

CHEMICAL METHODS

Saponification Number.[3]—The saponification number, or the Koettstorfer number, as it is sometimes called from the originator of the process, is *the number of milligrams of potassium hydroxide required to saponify 1 gram of the oil.*

Method.—Weigh 1.5 to 2.0 grams of the oil or melted fat into a 100-cc. Erlenmeyer flask. This is best done by weighing 5–10

[1] Grün and Schacht: *Ber.*, **1907,** 1778.

[2] Average values: for usual variations see Table XXIX, page 175.

[3] Koettstorfer: *Z. anal. Chem.*, **1879,** 199.

grams of the oil in a small beaker together with a small pipette or medicine dropper. The required amount of oil (8 drops = approximately 0.2 gram) is transferred by means of the dropper to the flask, taking care not to get any on the neck, the dropper replaced and the whole re-weighed. Add carefully from a pipette or burette 25 cc. of approximately half-normal potassium hydroxide,[1] close the flask with a cork carrying a straight glass tube several feet long, or place a small funnel in the neck of the flask, and heat on a boiling water-bath for 30 minutes or until completely saponified. When saponification is complete, as shown by a clear solution free from fat globules, cool the flask, add 1 cc. of phenolphthalein solution and titrate the excess of alkali with half-normal hydrochloric acid.

Two blank determinations must be carried out at the same time, using similar flasks, and taking all precautions as to draining of pipette or burette, time and condition of boiling, etc., in order that a true correction may be made.

Since each cubic centimeter of half-normal acid is equivalent to 28.05 mg. of KOH, the *Saponification number* is found by subtracting the number of cubic centimeters of acid used in titrating the sample from the amount used for the blank, multiplying the result by 28.05 and dividing by the weight of oil taken.

Notes.—The saponification number, as just described, should not be confounded with the "Saponification equivalent"[2] a term occasionally employed, which is the number of grams of oil which would be saponified by a liter of normal alkali.

The purest alcohol obtainable must be employed in the preparation of the alcoholic potash since ordinary alcohol contains an appreciable amount of aldehyde, which in the presence of the potash forms the yellow aldehyde resin and gradually darkens, so that it is difficult to see the end-point. For this reason, the removal of the aldehyde by silver oxide gives a much more satisfactory solution. If the saponified oil is still too dark to titrate, better results may be secured by diluting it with 50 cc. of *neutral* alcohol or by using with the phenolphthalein an equal amount of a

[1] Dissolve 40 grams of "potash by alcohol" in 1 liter of alcohol which has been purified by distilling over potash or preferably by treatment with silver oxide. (See Dunlap: *J. Am. Chem. Soc.*, **1906**, 397.)

[2] Allen's Com. Org. Anal., 4th Ed., Vol. II, p. 16.

cold-saturated alcoholic solution of Alkali Blue 6B,[1] (red with alkalies, blue with acids).

The saponification and titration should be carried out with as little access of air as possible, since the alkali solution readily absorbs carbon dioxide, a still further reason for making the blanks precisely parallel to the determination.

The saponification number is, as would be expected, inversely proportional to the mean molecular weights of the fatty acids present, its principal value being, as a matter of fact, to indicate the presence of the lower fatty acids such as occur in cocoanut oil, butter, etc. The following table[2] shows the differences found in the common glyceryl esters of the edible oils:

TABLE XXIII.—SAPONIFICATION VALUES OF GLYCERYL ESTERS

Butyrin	557.3
Laurin	263.8
Palmitin	208.8
Stearin	189.1
Olein	190.4
Erucin	160.0
Linolein	191.7
Arachidin	172.7

Since the natural oils contain more than one ester and these by no means in a pure state, the values actually obtained in practice do not show such differences as the above table might indicate. The test is, however, of distinct value in showing the presence of glycerides of the "butter acids" or of unsaponifiable matter.

If from the saponification number be subtracted the *acid number* (see below) the difference will be the number of milligrams of alkali actually used in the saponification of the glyceryl esters, the so-called *ester number*.

The values of the common edible oils are found in the table on the following page.

Free Fatty Acids.—Weigh 10–20 grams of the oil or fat into an Erlenmeyer flask, add 50 cc. of 95 per cent. alcohol which has been previously carefully neutralized to phenolphthalein with tenth-normal sodium hydroxide, heat on the water-bath nearly

[1] Marcusson: *Z. angew. Chem.*, **1911,** 1297.

[2] Allen: Org. Anal. (4) II, 17; Sherman: Org. Anal., p. 146.

TABLE XXIV.—COMMON EDIBLE OILS IN ORDER OF THEIR SAPONIFICATION VALUES[1]

Cocoanut oil	253	Mutton tallow	193
Butter fat	227	Almond oil	191
Palm oil	200	Sesame oil	191
Cocoa butter	197	Sunflower oil	191
Lard	197	Corn oil	191
Lard oil	195	Olive oil	190
Beef tallow	195	Peanut oil	190
Cotton seed stearin	194	Rape oil	174
Poppyseed oil	193	Mustard oil	173
Cotton seed oil	193		

to boiling, and titrate with tenth-normal alkali and phenolphthalein. It is necessary to shake thoroughly after each addition of alkali to secure complete extraction of the fatty acid from the immiscible oily layer.

If the solution is dark-colored Alkali Blue 6B may be used in place of phenolphthalein as suggested in the preceding method.

The result may be expressed as *percentage of oleic acid* (1 cc. $\frac{N}{10}$ alkali = 0.0282 gram of oleic acid) or as the milligrams of potassium hydroxide required to neutralize the free fatty acids in 1 gram of oil (*acid number*).

Note.—The presence of free fatty acids is due ordinarily to decomposition of the glycerides caused by chemical treatment or bacterial action, accelerated by light and heat. The amount found in edible fats and oils is not very considerable, although in palm oil the quantity present may be as great as 75 per cent. (calculated as palmitic acid). The determination is often used to distinguish between edible and non-edible olive oil, for fixing the duty.

Iodine Number.—The iodine number is *the number of grams of iodine absorbed by* 100 *grams of the oil.*[2] This constant is perhaps the most valuable of the general methods used for differentiating or identifying oils, in that it readily serves to indicate the group to which the oil belongs and is not so easily affected by slight changes in the oil as are some of the other constants.

The method depends upon the fact pointed out on page 148,

[1] Average values; for usual variations see Table XXIX, page 175.

[2] Although expressed as iodine the substance actually absorbed is iodine chloride or iodine bromide, depending on the method employed.

that, unlike the fatty acids of the acetic series, the unsaturated acids of the oleic or linoleic series,[1] as well as their glyceryl esters, absorb halogens to form mainly addition products. Thus oleic acid, $C_{17}H_{33}COOH$, takes up two atoms of iodine and forms the *addition product*, di-iodo stearic acid, $C_{17}H_{33}I_2COOH$.

Iodine itself is absorbed very slowly by the fat or oil, hence the reaction is ordinarily carried out in such a manner that the addition shall take place through the agency of iodine chloride or bromide. The method most widely used in the past and one which still finds considerable application, is that of Hübl,[2] based upon the use of an alcoholic solution of iodine in the presence of mercuric chloride. A study of the mechanism of the Hübl process, however, led Wijs[3] to propose the use of iodine monochloride directly. This reagent, used in acetic acid solution, is more stable and acts more quickly than the Hübl reagent. Subsequently Hanus[4] suggested the use of iodine bromide instead of the chloride, as being easier to prepare.

Both the Wijs and Hanus methods have advantages over the Hübl process and are gradually taking its place. The Wijs method is more commonly used in England and Germany and is stated by Lewkowitsch[5] to give results more nearly correct than the Hanus method. The latter, however, has been adopted as a provisional method by the Association of Agricultural Chemists and is considerably more convenient to use. In the case of the edible fats and oils, moreover, it gives entirely satisfactory results. Hence only the Hübl and Hanus methods will be taken up here. The Hübl method is discussed at considerable length because it shows the mechanism of the reaction more clearly than some of the later methods. For a detailed description of the Wijs method, reference may be made to Lewkowitsch (*loc. cit.*) or to Allen's Commercial Organic Analysis, 4th Ed., Vol. II.

Hübl Method.—*Reagents.*—(*a*) Iodine Solution. Dissolve 26 grams of iodine in 500 cc. of 95 per cent. alcohol. Dissolve also

[1] The acids of the still less saturated series, as linolenic and certain of the hydroxy acids also absorb halogens, but not occurring commonly in edible oils, are not discussed here.

[2] *Dingl. polyt. J.*, **1884,** 281.

[3] *Ber.*, **1898,** 750; *Z. Nahr. Genussm.*, **1898,** 561.

[4] *Z. Nahr. Genussm.*, **1901,** 913.

[5] Oils, Fats and Waxes (Ed. 5), Vol. I, p. 409.

30 grams of mercuric chloride in 500 cc. of similar alcohol, filtering the solution if not perfectly clear. Mix the two solutions in equal proportions for use. Since the solution loses considerable free iodine when first mixed it should be allowed to stand at least 12 hours before using. On the other hand, since the reagent gradually loses strength it is best not to use it after the second day. It is therefore best to keep the two solutions on hand and mix them in small quantities as wanted for use.

(*b*) Standard Sodium Thiosulphate Solution (approximately tenth-normal). Dissolve 25 grams of the recrystallized salt in a liter of water.

(*c*) Standard Potassium Bichromate Solution. Weigh out 3.8633 grams of pure potassium bichromate, dissolve in water and dilute to a liter. If the bichromate is pure and dry 1 cc. of this solution should be equivalent to 0.01 gram of iodine. The solution should be checked against iron wire, or a pure ferrous salt, containing a definite percentage of iron.

Standardizing the Thiosulphate Solution.—Measure 20 cc. of the bichromate solution into an Erlenmeyer flask, add an equal volume of water, 10 cc. of a 15 per cent. potassium iodide solution and 5 cc. of concentrated hydrochloric acid. Titrate with the thiosulphate until the red color, due to the free iodine, has changed to a pale yellow, then add 2 cc. of a freshly prepared starch solution (1 : 200) and titrate cautiously until the blue color changes to a sea-green.

The reaction may be expressed:

$$K_2Cr_2O_7 + 14HCl + 6KI \rightarrow 2CrCl_3 + 8KCl + 7H_2O + 3I_2$$

Process.—Weigh into a 300 cc. glass-stoppered bottle 0.2–0.4 gram of the sample in case of an oil, or 0.5–1.0 gram of a fat, and dissolve in 10 cc. of pure chloroform. Add 30 cc. of the iodine—mercuric chloride solution (50 cc. in the case of oils) from a pipette or glass-stoppered burette, taking care that none of the solution touches the neck of the bottle. Carefully insert the stopper, place a few drops of potassium iodide (15 per cent. solution) around the stopper to prevent loss of iodine, shake gently and allow the bottle to stand in a dark place for 3 hours. In similar bottles carry out two blank determinations in exactly the same manner and measuring the same quantity of reagents.

If during this absorption period the solution becomes nearly decolorized more of the mixed iodine solution must be added.

At the end of 3 hours carefully remove the stopper, add 20 cc. of the potassium iodide, pouring it over the stopper, and 100 cc. of water. If a red precipitate of mercuric iodide forms, add more potassium iodide. Titrate immediately with the standard sodium thiosulphate solution, which may be run in rapidly until the solution becomes pale yellow. Then add 2 cc. of the starch solution and titrate to the disappearance of the blue color. Toward the end of the titration stopper the bottle and shake vigorously in order to react with any iodine that may be dissolved in the chloroform. From the amount of thiosulphate solution employed, as compared with the blanks, calculate the percentage of iodine absorbed.

Example.—Weighed out 0.3978 gram of peanut oil; added 50 cc. of iodine solution. For titrating back used 32.55 cc. of a thiosulphate solution of which 15.20 cc. were equivalent to 0.2 gram of iodine. In a blank determination 25 cc. of iodine required 29.15 cc. of thiosulphate.

From blank, 50 cc. iodine solution = 58.30 cc. thiosulphate.

$$\begin{array}{r} 58.30 \text{ cc.} \\ 32.55 \text{ cc.} \\ \hline 25.75 \text{ cc.} \end{array}$$

0.3978 gram oil corresponds to 25.75 cc. thiosulphate.

$$25.75 \text{ cc. thiosulphate} = \frac{0.2 \times 25.75}{15.20} \text{ grams of iodine.}$$

Since this amount of iodine is absorbed by 0.3978 gram of fat.

$$100 \text{ grams would absorb } \frac{25.75 \times 0.2 \times 100}{0.3978 \times 15.20} = 83.1 \text{ grams.}$$

The iodine number, then, is 83.1.

Notes.—It will be readily apparent that the method as described is one requiring careful adherence to details in order to carry it out successfully. The chief points to be observed are:

1. *The strength of the iodine solution.* This rapidly deteriorates so as to be unsuitable for use within a few days. 30 cc. of the iodine solution should be equivalent to not less than 50 cc. of a tenth-normal thiosulphate solution.

2. *The excess of iodine present over that actually absorbed.* This should be at least 100 per cent. An excess of mercuric chloride is not essential, but it is necessary that at least one molecule be present for every two atoms of iodine.

3. *The time allowed for the reaction.* The greater part of the iodine is absorbed in 2 hours, and in the case of the edible oils, 3 hours is sufficient time to allow. With the drying oils, like linseed, 24 to 48 hours is usually required.

Care must be taken to prevent loss of iodine by volatilization when the sample stands so long, and Sherman[1] recommends the use of special flasks with a gutter which can be filled with potassium iodide solution.

When the titrated solution is allowed to stand it frequently becomes blue again, due probably to the splitting off of iodine from the compound formed, the reaction being to some extent a reversible one. The first end-point should be the one taken. The active substance involved in the Hübl process is undoubtedly iodine monochloride, which is formed when the iodine and mercuric chloride solutions are mixed, as expressed by the equation:

$$HgCl_2 + 2I_2 \leftrightarrows HgI_2 + 2ICl$$

It is probably the iodine monochloride which is absorbed by the fat, although from an analytical standpoint it is immaterial whether this or free iodine is the active agent, since upon adding potassium iodide an equivalent amount of iodine is liberated and titrated.

$$ICl + KI \rightarrow KCl + I_2$$

Thus in the blank determinations, the amount of free iodine titrated, since none has been removed, must be the amount present as free iodine in the original solution. A logical development of the conception of iodine monochloride as the active agent was the proposal of Wijs to use this directly, or the use of iodine bromide as suggested by Hanus.

Hanus Method.—*Reagents.*—(*a*) Iodine Solution.—Dissolve 13.2 grams of iodine in a liter of glacial acetic acid (99.5 per cent. strength). This is conveniently done by heating on the water-bath and adding the acetic acid in small portions. When cool add enough bromine to double the halogen content as shown by titration; 3 cc. is ordinarily sufficient.

(*b*) Potassium iodide, potassium bichromate and sodium thiosulphate solution as in the Hübl method, page 162.

Process.—Weigh into a 300-cc. glass-stoppered bottle about 0.5

[1] Organic Analysis, 2d Ed., p. 150.

gram of a fat, 0.25 gram of salad oil or 0.10 to 0.20 gram of a drying oil, and dissolve in 10 cc. of chloroform. Add 30 cc. of the iodine solution prepared as above and complete the method as described under the Hübl process, except that the time of standing should be 30 minutes, and only 10 cc. of the potassium iodide need be added instead of 20 cc. Blank determinations should be made at the same time and in the same manner. The thiosulphate solution is standardized as described on page 162.

Notes.—Care should be taken that the reagents are pure, especially the acetic acid, which should show no green color when warmed on the water-bath with potassium bichromate and sulphuric acid.

As in the Hübl method it is essential that a considerable excess of iodine, at least twice the amount absorbed, should be present. For this reason, when using a definite amount of the iodine solution, it is necessary to regulate accordingly the amount of oil or fat weighed.

It is best to measure out the various quantities of the iodine solution required for duplicate determinations and for the blanks within a short interval of time, since on account of the very high coefficient of expansion of acetic acid, the strength of the solution is materially altered by slight changes in temperature.

TABLE XXV.—COMMON EDIBLE OILS IN ORDER OF THEIR IODINE NUMBERS[1]

Poppyseed oil	136	Peanut oil	93
Sunflower oil	127	Olive oil	85
Corn oil	120	Lard oil	75
Cotton seed oil	110	Lard	58
Sesame oil	108	Palm oil	55
Mustard oil	104	Tallow	40
Rape oil	101	Cocoa butter	35
Almond oil	97	Butter fat	32
Cotton seed stearin	96	Cocoanut oil	9

For the purposes of food analysis the Hanus method is to be preferred to the Hübl, since the reaction is much more rapid and the solution decidedly more stable. With fats and oils having iodine numbers less than 100, the differences in the results obtained by the two method are no greater than the variations which

[1] Average values; for the usual variations see Table XXIX, page 175.

may be found in the oil itself. With oils of high iodine value the Hanus method gives distinctly higher results than the Hübl. With resins and drying oils the Wijs method is preferable. A comparison of the values given by the three methods on the common edible oils has been made by Tolman and Munson[1] and by Hunt.[2]

Acetyl Value.—The acetyl value is *the number of milligrams of potassium hydroxide required to neutralize the acetic acid obtained by saponifying* 1 *gram of an acetylated oil or fat.*

Process.[3]—(*a*) *Acetylation.*—Boil 10 grams of the oil or fat with twice as much acetic anhydride in a round-bottomed flask provided with a reflux condenser for 2 hours. Pour the resulting mixture into a large beaker containing 500 cc. of hot water and boil for half an hour. To prevent bumping pass a slow current of carbon dioxide into the liquid through a finely drawn out tube reaching nearly to the bottom. Allow the mixture to separate into two layers, siphon off the water and boil the oily layer with successive portions of fresh water until the wash water no longer reacts acid to litmus paper. Three boilings will ordinarily suffice. Prolonged washing might cause too low an acetyl value through slight dissociation of the acetyl product.

When washed free from acid, separate the acetylated fat carefully from the water in a separatory funnel and dry it in the water oven.

(*b*) *Saponification.*—Weigh out 2 to 5 grams of the dry acetylated fat and saponify with a measured amount of standard alcoholic potash exactly as in determining the saponification number (page 157). Evaporate on the water-bath to expel the alcohol, dissolve the soap in warm water and add standard hydrochloric acid exactly equivalent to the alcoholic potash used. Warm gently on the water-bath until the fatty acids form a layer on the top. Filter through a wet filter and wash the fatty acids with boiling water which has been previously boiled to ensure the removal of carbonic acid. Wash until the washings are no longer acid and titrate the filtrate and washings with tenth-normal sodium hydroxide and phenolphthalein.

[1] *J. Am. Chem. Soc.*, **1903**, 244.

[2] *J. Soc. Chem. Ind.*, **1902**, 454.

[3] Benedikt: Analyse der Fette und Wachsarten; Lewkowitsch: *J. Soc. Chem. Ind.*, **1897**, 503.

In a separate determination weigh 2–5 grams of the original oil or fat and carry out the saponification part of the process exactly as described above. Subtract the amount of alkali required to titrate the soluble fatty acids in the filtrate from the amount used for a corresponding quantity of the acetylated oil as found above. The difference, multiplied by 5.61[1] and divided by the weight in grams of acetylated fat taken, is the *acetyl value.*

Notes.—The method is based on the principle that when the hydroxy acids are heated with acetic anhydride the hydrogen of the hydroxyl group is replaced by the acetyl group. Thus in castor oil, consisting mainly of the glyceride of ricinoleic acid, the typical reaction might be expressed:

$$\underset{\text{Ricinoleic acid}}{C_{17}H_{32}(OH)COOH} + \underset{\substack{\text{Acetic}\\ \text{Anhydride}}}{(C_2H_3O)_2O} \rightarrow \underset{\text{Acetyl-ricinoleic acid}}{C_{17}H_{32}(O - C_2H_3O)COOH} + \underset{\text{Acetic acid}}{CH_3COOH}$$

While the acetyl value is in general a measure of the hydroxy acids present, it should be noted that it is affected by free alcohols, as phytosterol or cholesterol, as well. These, however, are found in only slight amount in edible fats and oils. Castor oil is unique in its high acetyl value, about 150, due to its high content of ricinolein; the values for the other fats and oils vary from 3–15.

The amount of soluble fatty acids also affects the acetyl value unless they are separately determined and a correction applied as is done above.

TABLE XXVI.—COMMON EDIBLE OILS IN ORDER OF THEIR ACETYL VALUES

	Average value		Average value
Castor oil	150.0	Cocoanut oil	6.6
Rape oil	14.7	Beef tallow	5.6
Cotton seed oil	13.0	Butter fat	5.2
Corn oil	11.2	Palm oil	5.1
Olive oil	10.6	Cocoa butter	2.8
Peanut oil	9.6	Lard	2.6

With oils containing a high percentage of volatile and soluble

[1] 1 cc. $\frac{N}{10}$ KOH = 5.61 mgm.

acids the error thus produced is considerable as shown in the following results obtained by Lewkowitsch.[1]

Oil or fat	Apparent acetyl value	Corrected acetyl value
Corn	8.7	5.8
Olive	12.9	10.7
Cotton seed	24.9	15.6
Lard	9.3	2.6
Cocoanut oil	23.2	2.3
Butter	45.2	1.9

The acetic acid being volatile with steam can be separated from the fatty acids by distillation, but the method is more tedious than the filtration process. In this case sulphuric acid rather than hydrochloric should be used to set free the fatty acids.

Maumené Number.—The Maumené number[2] is *the number of degrees centigrade which the temperature rises when* 10 *cc. of strong sulphuric acid is mixed with* 50 *grams of oil.* The exact strength of the acid employed is naturally of great importance and to secure more uniform results it has been proposed[3] to compare the rise in temperature with that produced by water under the same conditions, the latter being taken as 100. The number stated in this way is called the *specific Maumené number* or "specific temperature reaction." Thus, if 50 grams of cotton seed oil mixed with 10 cc. of sulphuric acid showed an increase in temperature from 22°C. to 97°C., its Maumené number would be 75; if 50 grams of water under the same conditions showed a rise of 46°, the specific Maumené number would be $\frac{75}{46} \times 100 = 163$.

Procedure.—Use a rather tall beaker of about 150 cc. capacity and insulate it by placing it in a larger beaker or agateware cup and packing the space between with cotton waste or felt. The insulation should be sufficient to prevent the outer vessel becoming perceptibly warm during a test. The oil to be tested, the water for the control test, and the sulphuric acid should all be at the same temperature, within a few degrees of 20°C. This may

[1] *Analyst*, **1899**, 319.

[2] Maumené: *Compt. rend.*, **1852**, 572.

[3] Thomson and Ballantyne: *J. Soc. Chem. Ind.*, **1891**, 234.

be brought about by immersing them in a tank of water at the desired temperature or more simply in most cases by letting them stand side by side on the laboratory desk for several hours.

Place 50 cc. of water in the beaker, immerse the thermometer and read the temperature to the nearest 0.1 degree. Add 10 cc. of the acid, running it slowly from a pipette and stir the mixture thoroughly with the thermometer. At intervals of a few seconds hold the thermometer in the center of the beaker and read the temperature. Record the highest point at which the thermometer remains constant for any appreciable time. As soon as this has been done throw out the mixture of water and acid in order not to heat the apparatus unnecessarily. When the initial temperature has been restored again repeat the determination. Duplicate determinations on water should not differ by more than 0.5°.

Having determined the rise of temperature with water, dry the beaker, weigh into it 50 grams of oil, within a drop or so, and carry out the test in exactly the same way as before, special care being taken to stir the mixture thoroughly if it becomes thick and gummy. Empty the beaker while still warm and wipe it thoroughly with cotton waste.

Notes.—The results obtained by this method are constant for a particular oil only when the details of manipulation are always rigidly observed, slight differences in the manner of carrying out the test causing serious discrepancies in the result obtained. For example, a difference of 5° has been noted when in one case the mixture was constantly stirred, and in another the oil was stirred until all the acid had been added and then the thermometer was held stationary in the center of the beaker. Much study has been given to the details of the method, especially with regard to the strength of the acid and the effect of diluting the mixture with some inert material. Since, however, the results reported by various authorities may differ quite considerably, having been obtained by slightly different methods, it is always best to compare a sample with one of known purity under exactly similar conditions.[1] In testing edible oils the strongest sulphuric acid

[1] See among others the following papers: Sherman, Danziger and Kohnstamm: *J. Am. Chem. Soc.*, **1902,** 266; Richter: *Z. angew. Chem.*, **1907,** 1613; Tortelli: *Chem.-Ztg.*, **1909,** 134, 171, 184; Wilisch: *Inaug. Diss.*, Augsberg, **1912.**

obtainable should be used; for fish and drying oils where the action is more violent, a more dilute acid may be employed or the oil may be diluted, using either a mineral oil or carbon tetrachloride, preferably the latter.

The results given by the Maumené test for different oils follow in general the iodine numbers, both being mainly dependent on the proportion of glycerides of the unsaturated fatty acids. An important difference, and one in which lies the greatest value of the Maumené number, is the fact that oils which have been oxidized by standing show an increased Maumené number, but a decreased iodine value. This is illustrated by the following figures:[1]

Oil	Iodine number	Specific Maumené number
Olive oil	83.8	100
Olive oil after exposure	77.3	127
Lard oil	73.3	106
Lard oil after exposure	56.2	141
Cotton seed oil	105.2	171
Cotton seed oil after exposure	90.2	217

The Maumené number might be especially helpful in cases like these in showing that the abnormal values obtained for certain of the "constants" were due not to adulterants but to a change in the oil itself.

TABLE XXVII.—COMMON EDIBLE OILS IN ORDER OF THEIR SPECIFIC MAUMENÉ NUMBERS[2]

Poppyseed oil	220	Rape oil	140
Corn oil	180	Peanut oil	125
Sunflower oil	167	Almond oil	110
Mustard oil	160	Olive oil	100
Cotton seed oil	155	Lard oil	90
Sesame oil	155		

Reichert-Meissl Number.—(Volatile fatty acids.)
Hehner Number.—(Insoluble fatty acids.)

[1] Sherman: Organic Analysis, 2d Ed., p. 211.

[2] Average values; for the usual variations see Table XXIX, page 175.

These two methods, although possibly of general application, are of greatest value in testing the purity of butter fat, and will therefore be described under the special methods for the analysis of butter fat on pages 204 to 210.

Examination of the Mixed Fatty Acids.—In cases where the examination of the oil or fat has not yielded sufficiently definite information, and a sufficient quantity of the sample is available, it may be of advantage to determine some of the constants of the fatty acids themselves. These correspond in a general way to the values determined on the individual oils, but in some cases, as with the melting and solidifying points, are more characteristic for the acids than for their glycerides.

Preparation of the Free Acids.—Saponify 25 grams of the oil by boiling with 20 cc. of potassium hydroxide solution (sp. gr. 1.4) and 20 cc. of alcohol. Use an Erlenmeyer flask provided with a cork carrying a straight glass tube to serve as a condenser and heat on the water-bath. Evaporate the alcohol and dissolve the pasty residue in several hundred cc. of hot water. Add an excess of hydrochloric or sulphuric acid and boil gently until the fatty acids form a clear oily layer at the top of the liquid. Siphon off the aqueous layer or separate by a separatory funnel and wash several times with hot distilled water until free from mineral acid. Separate as thoroughly as possible from the water and pour the melted fatty acids through a plaited filter in a funnel which is kept warm. On the fatty acids thus obtained the iodine number, melting point, or refractive index may be determined by the methods previously described.

Note.—To test the completeness of the saponification 3 cc. of the fatty acids are dissolved in 15 cc. of strong alcohol and 15 cc. of ammonia added. If an appreciable amount of fat has escaped saponification the mixture will become turbid. If the original fat contained much unsaponifiable matter turbidity might be due to that cause also. This will not usually occur, however, with edible fats and oils.

Solidifying Point of Fatty Acids.—*"Titer Test."*—The determination of the solidifying point of the mixed fatty acids is much more characteristic than the melting point, and is largely used for the commercial testing and valuation of fats. The method of

the Association of Official Agricultural Chemists,[1] which is a modification of the original procedure proposed by Dalican, gives results which are 0.2° to 0.3° higher than those obtained by the earlier process but are probably more accurate on account of the more thorough drying of the fatty acids.

(*a*) *Apparatus.—Standard Thermometer.*—The thermometer must be graduated in tenth degrees from 10° to 60°, with a zero mark, and have an auxiliary reservoir at the upper end, also one between the zero mark and the 10° mark. The cavity on the capillary tube between the zero mark and the 10° mark must be at least 1 cm. below the 10° mark, the 10° mark to be about 3 or 4 cm. above the bulb, the length of the thermometer being about 15 in. over all. The thermometer is annealed for 75 hours at 450°C., and the bulb is of Jena normal 16''' glass, moderately thin, so that the thermometer will be quick acting. The bulb is about 3 cm. long and 6 mm. in diameter. The stem of the thermometer is 6 mm. in diameter and made of the best thermometer tubing, with scale etched on the stem, the graduation to be clear cut and distinct, but quite fine.

(*b*) *Determination.*—Saponify 75 grams of fat in a metal dish with 60 cc. of 30 per cent. sodium hydroxide (36° Baumé) and 75 cc. of 95 per cent. by volume alcohol or 120 cc. of water. Boil to dryness, with constant stirring to prevent scorching, over a very low flame or over an iron or asbestos plate. Dissolve the dry soap in a liter of boiling water, and if alcohol has been used boil for 40 minutes in order to remove it, adding sufficient water to replace that lost in boiling. Add 100 cc. of 30 per cent. sulphuric acid (25° Baumé) to free the fatty acids, and boil until they form a clear, transparent layer. Wash with boiling water until free from sulphuric acid, collect in a small beaker, and place on the steam bath until the water has settled and the fatty acids are clear; then decant them into a dry beaker, filter, using hot-water funnel, and dry 20 minutes at 100°C. When dried, cool the fatty acids to 15° or 20°C. above the expected titer and transfer to the titer tube, which is 25 mm. in diameter and 100 mm. in length (1 by 4 in.) and made of glass about 1 mm. in thickness. Place in a 16-ounce saltmouth bottle of clear glass,

[1] *Bur. of Chem., Bull.* **107,** p. 135; Wolfbauer: *J. Soc. Chem. Ind.,* **1894,** 181.

about 70 mm. in diameter and 150 mm. high (2.8 by 6 in.), fitted with a cork, which is perforated so as to hold the tube rigidly when in position. Suspend the thermometer, graduated to 0.10°C., so that it can be used as a stirrer, and stir the mass slowly until the mercury remains stationary for 30 seconds. Then allow the thermometer to hang quietly, with the bulb in the center of the mass, and observe the rise of the mercury. The highest point to which it rises is recorded as the titer of the fatty acids.

Test the fatty acids for complete saponification as on page 171.

Note.—It is essential that the details of the process be strictly followed and especially that the fatty acids be dry. Duplicate determinations should agree easily within 0.1°; variations in the results obtained by different chemists are due mainly to differences in the method of stirring during the test.

TABLE XXVIII.—COMMON EDIBLE OILS IN ORDER OF TITER TESTS OF MIXED FATTY ACIDS[1]

	°C.		°C.
Cocoa butter	49.0	Cocoanut oil	23.0
Mutton tallow	45.0	Sesame oil	22.5
Beef tallow	44.0	Olive oil	20.0
Palm oil	43.0	Corn oil	19.0
Cotton seed stearin	38.0	Sunflower oil	17.5
Butter fat	35.5	Poppyseed oil	15.8
Lard	35.5	Rape oil	13.0
Cotton seed oil	33.0	Almond oil	11.6
Peanut oil	28.5		

Unsaponifiable Matter.—The amount of unsaponifiable matter, by which is meant all substances which may occur in fats that are insoluble or are incapable of forming soluble soaps with alkalies, is usually not over 1 to 2 per cent. in edible fats and oils if pure The presence of any appreciable quantity will be indicated if the solution of the soap in alcoholic potash be diluted with warm water by the appearance of oily drops or of a whitish cloud.

[1] Average values; for the usual variations see Table XXIX, page 175.

Determination.[1]—Weigh 3 grams of the fat or oil into a 150 cc. flask and boil for 1 hour under a reflux condenser with 25 cc. of alcoholic potash (see page 158). Transfer to a stoppered 100-cc. graduated glass cylinder and make up to 50 cc. with cold water. Add 30 cc. of redistilled petroleum ether (boiling point 35–50°C.) and shake vigorously. Draw off the petroleum ether layer by a siphon, or as described on page 114. Repeat the operation with four more portions of petroleum ether. Place the 150 cc. of petroleum ether in a 250 cc. separatory funnel and wash it three times with 20 cc. of 50 per cent. alcohol. Transfer the petroleum ether to a tared flask, evaporate (*away from a flame*) and dry the residue in the water-oven to constant weight.

Notes.—If the oil or fat has been extracted by ether or petroleum ether from a food product, the unsaponifiable matter may be considerable in amount and be of quite diverse character, including resinous substances, paraffin or mineral oils. If the oil or fat, on the other hand, is examined as such and is reasonably pure, the unsaponifiable matter is ordinarily quite insignificant in amount, consisting almost entirely of *cholesterol* or *phytosterol.*

These two substances are isomeric monatomic alcohols of the general formula $C_{27}H_{45}OH$, the former being characteristic of the animal fats, as the latter is of the vegetable oils. Still another alcohol, *sitosterol*, is isomeric with phytosterol and probably identical with it. The fact that they occur in the different classes of oils becomes at times of analytical importance when it is required to decide whether a given sample is of animal or vegetable origin, or whether a mixture of animal or vegetable fats or oils is present.

This can be done by noting the character of the crystals obtained by slow evaporation of the purified materials from alcohol,[2] or better in the case of mixtures by a determination of the melting point of the acetyl esters, the so-called "phytosteryl acetate test."[3]

[1] Spitz and Honig: *J. Soc. Chem. Ind.*, **1891**, 1039; *J. Ind. Eng. Chem.*, **1911**, 51.

[2] Bömer: *Z. Nahr. Genussm.*, **1898**, 31, 544; Lewkowitsch: *J. Soc. Chem. Ind.*, **1899**, 557; Zetsche: *Pharm. Centralh.*, **1898**, No. 49.

[3] Bömer: *Z. Nahr. Genussm.*, **1901**, 865, 1070; **1902**, 1018; Gill and Tufts: *J. Am. Chem. Soc.*, **1903**, 251, 254, 498.

TABLE XXIX.—CONSTANTS OF EDIBLE FATS AND OILS

Name	Specific gravity	Refractive index (15.5°C.)	Melting point, °C.	Saponification number	Iodine number	Maumené number	Spec. temp. number	Acetyl value	Reichert-Meissl number	Hehner number	Fatty acids		
											Melting point, °C.	Titer °C.	Iodine number
Almond oil	0.914–0.920	1.472–1.475	.....	188–195	93–100	51–54	95–120	.....	0.5	96.0	13.0–14.0	10.0–11.5	93.5–96.5
Butter fat	0.930–0.940	1.445–1.449[1]	28–35	221–233	26–38	.....	.....	1.9–8.6	25.0–33.0	86.5–89.5	38.0–42.0	34.0–37.0	28.0–30.0
Castor oil	0.960–0.967	1.479–1.481	.....	175–185	82–90	46–47	84–94	150.0	1.0–2.0	.....	13.0	3.0	87.0–88.0
Cocoanut oil	0.926–0.926	1.439–1.443[1]	22–27	246–260	8–10	.....	.....	2.8	0.2–0.8	94.5	48.0–52.0	48.0–49.0	33.0–38.0
Cod-liver oil	0.922–0.930	1.479–1.485	.....	180–190	135–175	113–116	240–280	0.9–12.3	6.6–8.4	82.5–90.5	24.0–27.0	21.0–25.0	8.4–9.0.0
Corn (maize) oil	0.921–0.927	1.475–1.477	.....	187–193	115–124	79–83	160–200	4.8	0.3–0.6	95.5	22.0–25.0	14.0–19.0	164.0–171.0
Cotton seed oil	0.920–0.925	1.473–1.476	.....	191–196	105–115	75–80	140–175	11.0–11.5	4.0–5.0	93.0	18.0–21.0	19.0	113.0–115.0
Cotton seed stearin	0.918–0.923	.....	26–40	194–195	89–103	48	.....	8.0–18.0	0.7–0.9	95.0–96.0	35.0–40.0	31.0–35.0	110.0–115.0
Lard	0.934–0.938	1.450–1.454[1]	36–45	195–200	50–65	25–35	.....	.....	.....	96.0	27.0–30.0	35.0–42.0	94.0–96.0
Lard oil	0.915–0.916	1.469–1.472	.....	193–198	67–82	47	80–100	2.6	0.2–0.6	93.0–95.0	35.0–46.0	33.0–38.0	63.0–66.0
Menhaden oil	0.925–0.931	1.479–1.482	.....	188–193	148–172	123–128	305–360	.....	00.0	97.0	.....	.....	95.0–109.0
Mustard oil	0.915–0.919	1.474–1.477	.....	171–176	94–113	42–44	140–180	.....	1.0	.....	.....	.....	.....
Olive oil	0.915–0.918	1.470–1.472	.....	185–196	79–90	42–46	90–110	10.6	0.6	95.0–96.0	15.0–17.0	.....	109.0–120
Palm oil	0.921–0.924	1.450–1.452[1]	27–43	196–204	53–57	.....	.....	1.9–8.4	0.8–1.9	95.0–96.0	20.0–27.0	17.0–23.0	86.0–90.0
Peanut oil	0.917–0.920	1.471–1.474	.....	186–194	85–100	49–56	105–140	9.06	0.5	94.5–97.0	47.7–50.0	40.0–45.0	52.0–58.00
Poppyseed oil	0.924–0.926	1.476–1.478	.....	190–196	133–139	86–88	200–240	.....	00.0	95.0–96.0	27.0–30.0	28.0–29.0	96.0–103.0
Rape (colza) oil	0.913–0.917	1.474–1.476	.....	170–179	97–105	50–60	130–150	14.7	0.0–0.6	95.0–96.0	20.0–21.0	15.4–16.2	139.0
Sesame oil	0.921–0.925	1.474–1.476	.....	188–193	103–112	63–70	140–175	.....	1.2	94.5–96.5	17.0–21.0	12.0–14.0	97.0–105.0
Sunflower oil	0.924–0.926	1.474–1.478	.....	188–194	120–135	68–75	167	.....	.....	95.0–96.0	24.0–31.0	21.0–24.0	109.0–112.0
Tallow (beef)	0.860[2]	1.449–1.452[1]	43–48	193–198	35–45	.....	.....	2.7–8.6	0.2–0.5	95.0–96.0	18.0–24.0	17.0–18.0	124.0–134.0
Tallow (mutton)	0.858–0.860[2]	1.451[1]	44–47	192–195	32–45	.....	.....	.....	0.3	95.0–96.0	43.0–47.0	43.0–44.0	40.0–43.0
											47.0–49.0	43.0–46.0	34.0–36.0

[1] At 60°C. [2] At $\frac{99°}{15.5°}$

Cholesteryl acetate melts at 113°C.; phytosteryl acetate at 128°C. Both of these tests require considerable experience in order to give reliable results, hence they are not described here. Full details of the best methods of making the tests will be found in Lewkowitsch: Oils, Fats and Waxes, 5th Ed., pp. 588–595.

Special Tests.—Besides the general methods described above there are numerous tests which are specific for certain oils, depending upon the color reactions of impurities or upon the presence of characteristic constituents. Some of the more important of these will be described under the typical samples which are discussed, olive oil and butter fat.

Olive Oil

As typical of edible oils olive oil has been selected. This is used in enormous quantities as a salad and table oil, especially in the case of the best flavored and highest grades. Large quantities of edible oil of a somewhat lower quality are used for packing sardines and similar fish. Inferior grades are used as burning oil, for lubricating, and in the textile industries and soap making.

Source.—Olive oil is prepared by expression or extraction from the fruit of the olive tree, *Olea europea sativa*. The best grades are obtained from Italy, Spain and other countries bordering on the Mediterranean, although considerable quantities are produced in southern California and Africa.

The olives are gathered just before they are ripe as the oil at that time is of the highest quality. The yield of oil is ordinarily from 40 to 60 per cent., although the fruit grown in California seldom yields over 25 per cent. The quality of oil obtained is variable, depending on the care used in picking and storing the fruit. The oil with the finest flavor, and hence most highly valued as an edible oil, is that obtained from ripe, hand-picked olives by using moderate pressure and is known as "virgin" or "sublime" oil. Lower grades, but still largely used as edible or salad oils, are obtained by a second pressing, after the residue has been ground and mixed with water. The oils obtained by further pressing the residue or by extraction with carbon bisulphide are not used as edible oils but for technical purposes.

Properties.—The value of olive oil, as is true of the edible oils in general, is largely dependent on its color, freedom from sediment, and above all, on the taste. The color of genuine oil may vary from very pale yellow to golden; the oils of lower grades, owing to the more extended treatment of the pulp, contain enough dissolved chlorophyll to impart a distinct greenish tinge. Cases have been reported in which the green color was due to the addition of copper salts. This may be detected by dissolving the oil in ether and shaking with dilute sulphuric acid. Copper, if present, will be found in the acid aqueous layer and can be detected or determined quantitatively by the usual methods. Slight variations in flavor are not of importance in the chemical examination although they may be the prime factors in the valuation of a sample.

Chemically, the principal constituents are the glycerides of oleic and palmitic acids together with a smaller proportion of linoleic acid. The amount of free fatty acids should be less than 0.5 per cent. in an edible oil although the technical oils may contain up to 25 per cent., depending on how long the oil is left in contact with the pulp during manufacture. The unsaponifiable matter, which varies in amount from 0.5 to 1.0 per cent., is phytosterol.

When the oil is cooled to about 2°C. it deposits palmitin as a white, granular solid, and if kept in an ice box for a week or so will in many cases become quite solid. Tolman and Munson[1] have pointed out that California olive oil shows a much less tendency to become solid than do the French and Italian oils.

Forms of Adulteration.—On account of its relatively high price and being in great demand, olive oil is very liable to adulteration with other oils. Even the lower grades of edible oil, which are not so readily salable on account of inferior flavor, may be, for that very reason, mixed with a cheaper, bland oil in order to disguise the unpleasant taste. The blending of lower grades or cheaper olive oil to extend the supply of a recognized high-grade oil, is adulteration in the strict sense of the term, but is unfortunately practically impossible of detection by analysis. More "Lucca" oil is undoubtedly exported under that name than can be produced in the whole province of Tuscany, but

[1] *Bur. of Chem., Bull.* **77,** p. 49.

the evidence is statistical rather than chemical and obviously inapplicable to individual shipments or brands.

The most common adulterants are cotton seed, peanut, sesame and lard oils. Less frequently employed are corn, rape, poppy-seed, sunflower, mustard and even cocoanut and fish oils. An arrangement of the common adulterants as regards their commercial value would be in about the following order, the highest priced being placed first: Olive, lard, peanut, poppy, rape, cotton seed, corn, fish oil.

Analytical Methods.—For the detection of these added oils both quantitative tests and when available qualitative reactions should be employed. Of the general quantitative methods which have already been described (pages 151 to 174) the most useful are the specific gravity or refractive index, the iodine number and saponification value. In the majority of cases it will be advisable to determine carefully at least three of these constants in addition to the specific qualitative tests which are described below under the individual adulterants. It is of course possible to use a mixture of oils so skillfully that the ordinary limits for olive oil shall not be exceeded, hence the actual clue to the adulteration may be furnished by the qualitative tests alone, but the evidence should in all cases be confirmed by a careful study of the quantitative results, both directly and in their relation to one another.

In doubtful cases an examination of the fatty acids, especially as regards their melting point and iodine number, will furnish valuable information.

A reasonably safe procedure to follow for the systematic examination of olive or "salad" oils would be to determine first the refractive index, then the iodine value and saponification number of the sample. A comparison of the values obtained in these three tests with those given in the table on page 175 should give a good idea whether the oil is presumably genuine or adulterated, and point in some cases to the probable adulterant. These tests should then be followed by the specific reactions for cotton seed, sesame and peanut oil as given below. It cannot be too strongly emphasized that with all of the color reactions and special tests simultaneous tests should be made also upon genuine olive oil and upon a mixture of olive and a

reasonable proportion of the oil in question. This is especially necessary if the reactions are being tried for the first time. If the quantitative results still appear abnormal and no adulterant has been shown, one of the less commonly used oils as lard, rape or fish oil may be present, or the possibility of the addition of heated cotton seed oil should not be overlooked. The nitric acid test (see below) and the taste and odor of the oil both cold and heated, should be tried, taking the precaution, as before, to employ the same tests on known mixtures in order to acquire the necessary experience for correct interpretation of the results. Study carefully the characteristics of genuine olive oil, as given below, and consult the authorities listed at the end of the chapter, especially Allen[1] and Lewkowitsch.[2]

Interpretation of Results

Characteristics of Genuine Olive Oil.—Olive oil is a typical non-drying oil, hence is characterized by low iodine and Maumené numbers, these being lower than would be found normally in any oil that would be used as an adulterant. Restricting the range of variation of the iodine number to that occurring in *edible* oils, this determination alone should show by a distinctly high value the presence of 5 per cent. or more of a drying oil like poppy, or 15 per cent. of cotton seed, rape or sesame oil. Peanut oil, being more nearly like olive in its iodine number, is not readily detected in this way.

The range of values given in Table XXIX, page 175, covers practically all that would be met in an edible olive oil, although higher or lower values would undoubtedly be found in commercial oils. An excellent idea of the extreme range found in edible olive oil of the kinds most largely sold in this country may be gained from the following table compiled from the results of Tolman and Munson.[3]

Possibly the most noticeable feature of these analyses is the rather wide range that certain of the so-called "constants," as the iodine number, Maumené number and melting point of the

[1] Commercial Organic Analysis, 4th Ed., Vol. II.
[2] Oils, Fats and Waxes, 5th Ed., Vol. II.
[3] *Bur. of Chem., Bull.* **77.**

fatty acids, exhibit in oils free from adulteration. This strengthens still further the statement already made that the quantitative results obtained in the examination of an oil should be considered with relation to one another as well as individually. For instance, an oil with an iodine number approaching the maximum given in the table for Californian oils might be a genuine product or a mixture of a pure oil of low iodine number with an adulterant which would tend to raise the value. In the former case, however, the oil would probably show also a high specific gravity, refractive index and specific temperature reaction, thus making the standard of comparison more rigorous and tending better to exclude adulterated samples. The California oils, in general, show higher iodine numbers and lower melting points of the fatty acids, than do the French and Italian oils.

TABLE XXX.—ANALYSES OF OLIVE OIL

Source		Specific gravity	Ref. index 15.5° C.	Maumené number[1]	Specific temperature reaction	Iodine number	Saponification number	M. pt. of fatty acids	Free fatty acid as oleic, per cent.
California oils of known purity (383 samples).	Max.	0.9180	1.4718	52.1	109.7	89.8	194.4	31.0	8.21[2]
	Min.	0.9162	1.4703	38.0	94.5	78.5	189.3	19.2	0.20
	Avg.	0.9170	1.4713	46.9	101.8	85.3	190.9	22.9	1.20
Italian oils of known purity (18 samples).	Max.	0.9180	1.4713	49.1	104.7	86.1	192.0	29.3	2.79
	Min.	0.9155	1.4705	39.6	95.6	79.2	189.6	21.6	0.57
	Avg.	0.9163	1.4709	44.9	99.1	81.6	190.9	25.5	1.11
California commercial oils, not adulterated (12 samples).	Max.	0.9177	1.4717	51.0	.....	86.5	194.9	26.2	3.96
	Min.	0.9152	1.4705	41.0	.....	79.2	190.5	24.5	0.29
	Avg.	0.9165	1.4710	45.5	.....	82.2	192.7	20.7	1.95
Italian commercial oils, not adulterated (56 samples).	Max.	0.9179	1.4712	48.8	108.4	84.5	196.6	30.4	5.30
	Min.	0.9151	1.4701	39.8	88.4	77.5	190.1	21.0	0.70
	Avg.	0.9161	1.4706	44.0	97.8	80.9	192.6	26.6	2.37
French commercial oils, not adulterated (61 samples).	Max.	0.9183	1.4713	51.5	114.4	85.0	195.3	30.8	3.63
	Min.	0.9150	1.4699	40.7	90.4	79.0	190.5	23.7	0.45
	Avg.	0.9166	1.4708	45.1	100.1	81.3	193.0	27.3	1.59

The Federal standard[2] for olive oil is:

Olive oil is the oil obtained from the sound, mature fruit of the

[1] Rise of temperature with water = 45°. [2] Next lower = 3.51 per cent.

[2] U. S. Dept. of Agriculture, Office of the Secretary, Circ. **19.**

cultivated olive tree (*Olea europæa L.*) and subjected to the usual refining processes; is free from rancidity; has a refractive index (25°C.) not less than 1.4660[1] and not exceeding 1.4680; and an iodine number not less than 79 and not exceeding 90. *Virgin olive oil* is olive oil obtained from the first pressing of carefully selected, hand-picked olives.

Detection of Possible Adulterants of Olive Oil.—(1) *Cotton seed Oil.*—The edible grades of cotton seed oil, varying in color from nearly colorless to a light straw, are employed in considerable quantities, either mixed with olive oil or as a substitute under the name of "salad oil." In order that the oil shall not become turbid in cold weather, much of is it chilled, and the solid "stearin" filtered off and pressed. The cotton seed stearin thus obtained is used in lard and butter substitutes (see page 201). The term "stearin" is a misnomer, since practically no stearic acid is present in cotton seed oil, the solid fatty acids consisting mainly of palmitic acid.

In general, the addition of cotton seed oil to olive oil would raise the specific gravity, iodine number, specific temperature reaction and the melting point of the fatty acids (titer test). Of these the last is especially characteristic (see Table XXIX, page 175). Some of these might fail to show the adulteration if a third oil, say lard oil, were skillfully added also, but if the examination were reasonably complete, such a mixture could hardly escape detection.

Qualitative Tests.—Cotton seed oil gives several fairly definite and characteristic color reactions, which are extremely useful as confirmatory tests or to differentiate the results of the quantitative examination, as in the mixture cited above. They should not, however, be considered absolutely decisive or relied on to the exclusion of the analytical constants. Of these the two most useful are given below.

Halphen Test.[2]—To a 1 per cent. solution of sulphur in carbon bisulphide add an equal volume of amyl alcohol. Mix 3–5 cc. of the reagent and an equal volume of the oil to be tested in a test-tube and heat in a bath of boiling water, or better in boiling saturated brine, giving a temperature of about

[1] Corresponding values at 15.5°C. are 1.4625 and 1.4645.

[2] Halphen: *J. Pharm. Chim.*, **1897**, 390.

105°C. Continue the heating for 2 hours unless a color develops sooner. A reddish color is developed in the presence of cotton seed oil, being more intense and more rapidly produced the greater the proportion of cotton seed oil present.

Notes.—This test is undoubtedly the most delicate for cotton seed oil, as little as 1 per cent. being detected easily within the limitations given below. Cotton seed oil from different sources varies somewhat as regards the intensity of the reaction, but it has never been reported as given by any genuine olive oil.

The exact nature of the substance producing the red color is not definitely known[1] but it is present in very small amount and is destroyed or removed with relative ease.

The production of a red color cannot be taken as conclusive evidence of the presence of cotton seed oil since kapok oil, from the seeds of a tropical tree related to the cotton plant, and baobab oil give a similar reaction, the latter even stronger than cotton seed oil. Milliau[2] recommends the following procedure to distinguish them:

Saponify the oil, set free the fatty acids, wash and dry. Mix 5 cc. of the melted acids with 5 cc. of a 1 per cent. solution of silver nitrate in absolute alcohol, shake and allow to stand without heating. The presence of 1 per cent. or more of kapok or baobab oil causes a dark brown color in 20 minutes, while with cotton seed oil there is no reducing action unless the mixture is warmed.

It has also been shown that lard, lard oil or butter fat obtained from animals which have been fed on cotton seed meal will give the Halphen test even though no cotton seed oil has been added directly.

On the other hand, a negative test is no proof of the absence of cotton seed oil. Oil which is very old and rancid, or which has been heated to 250°C., for 10 to 20 minutes, or even to 200°C. for an hour, no longer gives the Halphen reaction. It is true that the heating of the oil gives it a disagreeable taste so that it would be less likely to be mixed with an edible oil. If then, the

[1] See among others Raikow: *Chem.-Ztg.*, **1900**, 562, 583; **1902**, 10; Gill and Denison: *J. Am. Chem. Soc.*, **1902**, 397; Rupp: *Z. Nahr. Genussm.*, **1907**, 74.

[2] *Compt. rend.*, **1904**, 807.

analysis points toward the presence of cotton seed oil and no positive result is obtained in the Halphen test, the reaction given below should be tried.

Nitric Acid Test.—Five cc. of the oil, which should be at room temperature, are shaken vigorously with an equal volume of nitric acid (sp. gr. 1.375) and allowed to stand. Cotton seed oil, and other seed oils as well, gives a deep brown color, usually within a few minutes, occasionally only after standing for some hours. Genuine olive oil, if reasonably fresh, gives no color, or at most a greenish yellow.

Notes.—This test, on account of the misleading conclusions which may be drawn from it, is one which must be applied with great caution. Comparison tests should always be made at the same time with pure olive oil and with mixtures of olive and cotton seed oils. Results should not be considered positive unless a high iodine number or some other quantitative confirmation is found. All cotton seed oils do not give the reaction with equal facility, and on the other hand, some other seed oils respond nearly as well as cotton seed. Archbutt[1] cites a sample of rape oil which when mixed with four times as much olive oil gave in 40 minutes a brown color not distinguishable from that produced by a similar proportion of cotton seed oil.

The test is distinctly less delicate and characteristic than the Halphen reaction.

2. *Peanut Oil.*—Peanut oil (called also *arachis* oil) occupies the same importance in European countries as an adulterant of olive oil that cotton seed oil does in this country. Apart from the fact that the refined oil, being practically colorless and tasteless, is admirably suited for admixture with olive oil, its analytical characteristics so clearly resemble those of olive oil that considerable quantities of it can be added to the latter without being detected by the usual quantitative figures. The iodine number is somewhat higher, but still a large proportion could be added to an olive oil of low iodine value and escape detection. The only successful method for showing its presence consists in the separation and identification of the characteristic arachidic acid, of which peanut oil contains approximately 5 per cent.

[1] Allen's Com. Org. Anal., 4th Ed., Vol. II. p. 139.

Detection of Arachidic Acid.—Bellier's[1] Method.—This is recommended as a simple qualitative test by Archbutt[2] and the writer has found it very satisfactory.

The necessary reagents are:

Alcoholic potash, made by dissolving 8.5 grams of pure potassium hydroxide in 70 per cent. alcohol and making up to 100 cc.

Acetic acid of such strength that 1.5 cc. will neutralize 5 cc. of the alcoholic potash. (Acid having a specific gravity of 1.04 is approximately correct.)

Procedure.—Weigh 1 gram of the oil into a dry test-tube, add 5 cc. of the alcoholic potash and boil gently over a small flame, avoiding evaporation as much as possible. Boil until the oil is completely saponified, which will take a little over 2 minutes. Add 1.5 cc. of acetic acid, or just sufficient to neutralize the alcoholic potash (use phenolphthalein as an indicator), mix well, cool rapidly by placing the test-tube in water at 17° to 19°C. and leave in the water at the stated temperature for not less than 30 minutes, shaking occasionally. Then add 50 cc. of 70 per cent. alcohol containing 1 per cent. by volume of hydrochloric acid (sp. gr. 1.16), shake well and again place in water at 17° to 19° for an hour. If no peanut oil be present the liquid will remain clear or at most slightly opalescent; with 10 per cent., or more, of peanut oil a flocculent, crystalline precipitate forms.

Note.—The method depends, as does also the Renard test given below, on the relative insolubility of arachidic acid in cold alcohol as compared with its lower homologues, palmitic and stearic acids.

Low-grade olive oils, which contain considerable unsaponifiable matter derived from the shell of the olive pit, may give a flocculent precipitate in this test although no peanut oil is present. This is not likely to be the case, however, with edible oils.

Renard Test.[3]—Weigh 20 grams of oil into an Erlenmeyer flask and boil with 200 cc. of alcoholic potash (40 grams of potassium hydroxide per liter of alcohol) until saponified. Neu-

[1] *Ann. Chim. Anal.*, **1899**, 4.

[2] Allen's Com. Org. Anal., 4th Ed., Vol. II, p. 99.

[3] Renard: *Compt. rend.*, **1871**, 1330; Tolman: *Bur. of Chem., Bull.* **107**, p. 145.

tralize with dilute acetic acid (sp. gr. 1.04), using phenolphthalein as an indicator, and pour gradually into a 500-cc. flask containing a boiling mixture of 100 cc. of water and 120 cc. of 20 per cent. lead acetate solution.

Boil for a minute and then cool the mixture by immersing the flask in cold water, preferably ice water, whirling the flask from time to time so that the precipitated lead soaps shall stick to its sides. When thoroughly cooled pour out the excess of lead acetate solution and wash the soap with cold water and then with 90 per cent. alcohol, draining as thoroughly as possible. Add 200 cc. of ether, cork the flask and allow to stand, shaking occasionally, until the soap is disintegrated, after which heat gently to boiling on a steam bath or electric heater, using a reflux condenser. (*Avoid the use of a flame!*) Boil for 5 minutes, then cool to 15°–17°C. and let stand over night in an ice chest.

Filter, wash the insoluble soaps thoroughly on the filter with ether, then wash them back into the flask with a stream of hot water acidified with hydrochloric acid. Add a considerable excess of dilute hydrochloric acid and enough hot water to give a volume of about 250 cc., and heat on the water-bath or over a small flame until the soap is entirely decomposed and the fatty acids separate as a clear oily layer. Frequent shaking will hasten the process, which usually requires about an hour. Nearly fill the flask with hot water, and when the fatty acids have entirely separated stand the flask in cold water until they form a solid cake. Remove the cake of fatty acids, drain, then return to the empty flask, fill up with hot water and repeat the process. After the second washing dissolve the cake of acids in 100 cc. of boiling 90 per cent. (by volume) alcohol. Cool to 15°C. and keep at this temperature with frequent shaking as long as any acid continues to crystallize out.

Filter into a graduated cylinder, wash the crystals twice with 10 cc. of 90 per cent. alcohol, noting the total volume of filtrate and washings, then wash several times with more dilute alcohol (70 per cent.), in which arachidic acid is only very slightly soluble. Make a hole in the filter, wash off the precipitate with boiling absolute alcohol into a tared dish, evaporate on the water-bath, dry and weigh. Correct the weight for the arachidic acid

which remains dissolved in the filtrate and washings by adding 0.0025 gram for each 10 cc. of 90 per cent. alcohol collected in the cylinder if the crystallization were carried out at 15°C., and 0.0045 gram for each 10 cc. if done at 20°C. The approximate weight of peanut oil (although probably too low) is obtained by multiplying the weight of arachidic acid by 20. Determine the melting point of the arachidic acid obtained, using the method described on page 154.

Notes.—The melting point of the "arachidic acid" obtained, which is in reality a mixture of arachidic and lignoceric acids, is somewhat variable, depending upon the care with which the process has been carried out, but should not be appreciably below 71°–73°C. The melting points of the pure acids are distinctly higher than this (77° for arachidic and 80.5° for lignoceric acid), the lowering being due to impurities.

It is essential to determine the melting point of the crystals, since cotton seed and lard oil have been found to give crystals resembling those of arachidic acid but having a considerably lower melting point.[1]

The separation of the lead soaps by ether (see page 149) is advantageous since in this way the acids of the oleic series, which interfere with the crystallization of the arachidic acid, are removed as their soluble lead salts, allowing the detection of as little as 5 or 10 per cent. of peanut oil.

In testing the purity of olive oil contained in manufactured food products allowance must be made for the fact that peanut oil, on account of the better flavor it is supposed to give, may be used during the process and a small portion may be present in the final product. Thus in olive oil from canned sardines, the presence of peanut oil in quantities not exceeding 5 per cent. is not regarded as an adulteration, since the fish are frequently cooked in peanut oil, drained, and then packed in olive oil.

Peanut oil contains from 3.5 to 5.5 per cent. of arachidic acid according to different observers. The only other oils which contain any appreciable amount are rape and mustard seed, and in these the quantity is too small to have much analytical significance. Olive oil, with the possible exception of some African oils, contains only a trace.

[1] Tolman and Munson: *Bur. of Chem., Bull.* **77**, p. 35.

According to Tortelli and Ruggieri,[1] the amount of peanut oil may be determined approximately by noting the temperature at which the separation of arachidic acid begins, as shown in the following table:

Temperature at which crystals begin to form, °C.	Per cent. peanut oil
35–38	100
31–33	60
28–30	50
25–26	40
22–24	30
20.5–21.5	20
18–20	10
16–17	5

3. *Sesame Oil.*—Sesame oil is obtained from the seeds of the sesame plant, grown chiefly in India, Egypt and the countries bordering on the Mediterranean, and is prepared in large quantities in France as an edible oil. It is used either alone or mixed with other oils and in certain European countries its use is compulsory in the manufacture of oleomargarine. Its pleasant flavor and freedom from odor make it a natural adulterant for the more expensive olive oil.

As in the case of cotton seed oil, the specific gravity, refractive index, Maumené number and iodine number of sesame oil are distinctly higher than for olive oil and serve to indicate its pressure when added in considerable quantities. Like cotton seed oil also, definite color reactions are characteristic of sesame oil and highly important analytically. Of these the best are the two following:

Baudouin's Test.[2]—Dissolve 0.1 gram of cane sugar in 10 cc. of hydrochloric acid (sp. gr. 1.20), add 20 grams of the oil to be tested, shake thoroughly for 1 minute and allow to stand. If 1 per cent. or more of sesame oil is present the aqueous layer will be colored red. Carry out at the same time a check experiment on an oil of known purity.

Notes.—Some genuine olive oils, especially those from Tunis and Algeria, give a pink color which might be attributed to a small percentage of sesame oil, but comparison with a sample

[1] *Chem.-Ztg.*, **1898**, 600.
[2] *Z. angew. Chem.*, **1892**, 509.

of genuine sesame oil will usually show the difference. This is said to be due to a coloring matter derived from the pulp of the olive and in doubtful cases the test should be made upon the liquid fatty acids rather than the oil itself, since the interfering substance does not pass into the acids. If a distinct quantity of sesame oil is present the red color will be observed in both the oily and aqueous layers.

The reaction is attributed to one of the constituents of the unsaponifiable part of the oil, a phenolic body[1] to which the name "sesamol" has been given. Other substances, such as vanillin and oil of cloves, will give a similar reaction, hence caution should be used in applying the test to oil extracted from confections or food products of mixed composition.

Villavecchia and Fabris' Test.—Villavecchia and Fabris found that the active agent in the preceding test was the furfural produced by the action of the hydrochloric acid on the sucrose, and they have modified the procedure by substituting an alcoholic solution of furfural for the sugar.

To 10 cc. of the oil to be tested add 3 drops of a 2 per cent. alcoholic solution of furfural and 10 cc. of strong hydrochloric acid, and shake the test-tube containing the mixture for half a minute. The aqueous layer will be colored red if sesame oil is present.

Note.—Furfural itself gives a violet color with hydrochloric acid, hence only a very small amount should be used.

4. *Corn Oil.*—Corn oil (maize oil) is produced in large quantities from the germs of Indian corn, *Zea mays*. Although considerably cheaper than olive oil, it has not in the past been largely used to adulterate the latter on account of its objectionable flavor. The modern refined oil, however, is nearly odorless and tasteless and hence a possible adulterant which should be taken into consideration in the analysis of salad oils.

By reference to the table on page 175, it will be seen that for corn oil the specific gravity, Maumené number, refractive index and iodine number are all decidedly higher than for olive oil, the last three values being even higher than for cotton seed oil. The high Maumené number and iodine number would render an ordinary olive oil suspicious if even only 10 to

[1] Kreis: *Chem.-Ztg.*, **1903**, 316.

15 per cent. of the adulterant were added. A mixture of corn oil with lard oil might be used which would simulate quite closely olive oil so far as the above constants are concerned, but with such a mixture the difference between the specific temperature reaction and the iodine number would still be much greater than with pure olive oil.[1] Further, the presence of the lard oil would be revealed by its characteristic odor when heated and possibly the phytosteryl acetate test. (See page 174.)

With any except the most highly refined corn oil, its presence would be revealed by the characteristic "grainy" odor and taste. There is no specific color reaction for this oil, although Tolman and Munson[2] state that nitric acid (specific gravity 1.37) when shaken with corn oil gives a peculiar red color which is quite different from the color given with cotton seed oil. If this test is employed, however, it should be made strictly comparative and the results should be interpreted with great caution.

The Reichert-Meissl number of corn oil has been found by several observers to be considerably higher than olive oil or its ordinary adulterants, and might prove of some value in detecting its presence.

5. *Poppyseed Oil.*—This oil is pressed from the seeds of the oriental or opium poppy, *Papaver somniferum*. The best grades, having almost no odor and a pleasant flavor suggestive of almonds, have been largely used in Europe as a salad oil and to adulterate olive oil.

What was said on page 188, under corn oil, regarding the raising of most of the constants of olive oil by its addition, applies with even greater force to poppyseed oil. The specific gravity, Maumené number, refractive index and iodine number are all exceptionally high and unless masked by the use of lard oil, would serve to indicate the adulteration.

6. *Lard Oil.*—The liquid portion or olein of lard, obtained by subjecting it to hydraulic pressure, is sold as *lard oil*. The winter-pressed oil, especially if made from edible grades of lard, and well refined, is entirely free from the odor and taste of lard, being a bland oil, nearly colorless to light yellow, and

[1] Sherman: Organic Analysis, 2d Ed., p. 183.
[2] *Bur. of Chem., Bull.* **77**, p. 37.

admirably suited for the adulteration of olive oil. Archbutt[1] states that "Superfine Lucca Oil" sometimes contains as much as 70 per cent. of lard oil.

Further, analytically it much resembles olive oil, the specific gravity, refractive index, Maumené number and saponification number being practically identical with the values given by olive. Its iodine number is decidedly lower than for olive oil although for purposes of adulteration this can hardly be regarded as a disadvantage, enabling the oil to be added in large proportion to olive oils of high iodine value or to be mixed with other oils as cotton seed or peanut, thus lowering the iodine number of the mixture to a point corresponding to that of olive oil.

For the detection of lard oil reliance must be placed chiefly upon the odor, the high melting point of the fatty acids and the presence of cholesterol.

Although lard oil carefully refined has very little odor when cold, it is intensified on heating the sample, and in many cases will serve for its detection if present in appreciable quantity. The analyst must of course first familiarize himself with the odor of lard oil itself, which is very similar to that of heated lard, and should experiment with mixtures of olive and lard oils, before drawing conclusions from the odor of an unknown sample.

The melting point of the mixed fatty acids, separated and determined as described on pages 171 and 172, is much higher than with olive oil, 21°–30°C. in one case and 33°–38°C. in the other. This difference, however, loses some of its analytical value, since an olive oil of high iodine number, which is the kind of olive oil with which lard oil would be most likely mixed, would naturally have at the same time a low melting point for the fatty acids, so that a considerable proportion of lard oil could be added without making the melting point abnormal.

In cases of doubt it may be necessary to show the presence of cholesterol, this being characteristic of the animal oils as phytosterol is of the vegetable oils. The method most advisable to follow is that of Bömer (see page 174), and working details of the procedure, together with many helpful hints, will be found in Lewkowitsch's Oils, Fats and Waxes.

7. *Fish Oil.*—The effect that might be produced on the con-

[1] Allen's Com. Org. Anal., 4th Ed., Vol. II, p. 112.

stants of olive oil by an admixture of fish oil, should be given consideration for two reasons. In the first place, fish oil, especially menhaden oil, is said to be considerably employed as an adulterant, and second, because many varieties of small fish, as sardines, are preserved in olive oil. The olive oil in this case will always contain a proportion of the body oil of the fish and some of its constants will be distinctly altered.

Menhaden oil, which may be taken as a type of the fish body oils, would be easily detected if added to olive oil in any large quantity, since its specific gravity, refractive index, Maumené number and iodine number are all so much higher than olive. It would of course be much harder to find if lard oil were added at the same time. For the same reason higher constants must be expected in the case of olive oil taken from cans of preserved fish.

In addition to the raising of the constants, fish oil would in many cases be identified specifically by its characteristic "fishy" odor, which is more pronounced upon heating. Fish oils contain also cholesterol, which can be separated and identified by its crystalline form or by the melting point of its acetyl ester. (See lard oil above, on page 190.) More easily carried out is the test given below.

Insoluble Bromide Test.—*Qualitative.*[1]—Dissolve in a test-tube about 6 grams of the oil in 12 cc. of a mixture of equal parts of chloroform and glacial acetic acid. Add bromine drop by drop until a slight excess is indicated by the color, keeping the solution cool by immersing the tube in a bath of water at about 20°C. Allow to stand 15 minutes or more and then place the test-tube in boiling water. If only vegetable oils are present, the solution will become perfectly clear, while fish oils will remain cloudy or contain a precipitate due to the presence of insoluble bromides.

Notes.—The method depends on the fact that the bromides of the unsaturated acids of vegetable oils, mainly linolenic acid, are completely soluble in the boiling mixture of chloroform and acetic acid, while the bromides of the somewhat different acids of fish

[1] Eisenschiml and Copthorne: *J. Ind. Eng. Chem.*, **1910**, 43; *Bur. of Chem., Bull.* **137**, p. 87.

oil remain undissolved. (See also the notes under the "Hexabromide Test" below.)

Any cloudiness or precipitate obtained in the cold, before the solution has been heated, should be disregarded, since the method depends absolutely upon the behavior of the bromides in the hot solution. If the oil is absolutely clear at the beginning of the test, it will remain so at the boiling temperature and any turbidity can be taken as indicative of fish oil. Five per cent. of fish oil in olive oil can be detected with certainty.

Quantitative Determination.—The "Hexabromide Test."[1]—Dissolve 1 to 2 grams of the oil in 40 cc. of ether to which 2–3 cc. of glacial acetic acid have been added, cool the solution to 5°C., and add bromine drop by drop from a pipette until a permanent brown color remains. Allow the solution to stand packed in crushed ice and in an ice box for at least 3 hours, filter through a Gooch crucible, keeping the main bulk of the precipitate from the filter until the end, wash four times with ice cold ether, dry the precipitate in the water-oven and weigh.

If a suitable centrifuge is available[2] it will be of advantage in removing the difficulties which attend the filtration of the gummy precipitate of insoluble bromides from the very volatile solvent. The process may be carried out in light test-tubes, which can be whirled in the centrifuge and the separated and washed bromides dried and weighed directly in the tubes.

Notes.—The test is not characteristic of fish oils in that it is given also by linseed oil, the reaction being due in the latter case to the unsaturated linolenic acid $C_{18}H_{30}O_2$, while with the fish oils it is usually attributed to the presence of jecoric acid, isomeric with linolenic. Lewkowitsch[3] however, disputes this view and states that the so-called jecoric acid is in reality an impure acid, clupanodonic, $C_{18}H_{28}O_2$, of the still less saturated series $C_nH_{2n-8}O_2$. In this case the compound formed would be *octobromoclupanodonin*, $C_3H_5(C_{18}H_{27}O_2Br_8)_3$ instead of the *hexabromolinolenin*, $C_3H_5(C_{18}H_{29}O_2Br_6)_3$. The fish oils can be distinguished from linseed in this test, however, by noting the be-

[1] Hehner and Mitchell: *Analyst*, **1893**, 313; Tolman: *J. Ind. Eng. Chem.*, **1909**, 341.

[2] Tolman: *Loc. cit.*

[3] Oils, Fats and Waxes, 5th Ed., Vol. I, p. 210.

havior of the insoluble bromides on heating. The bromides from linseed oil melt at about 144°C. to a *clear liquid*, while those from fish oils decompose before melting, forming a dark mass.

The weight of insoluble bromides obtained from fish oil average about 50 per cent., so that from the determination an approximation of the amount of fish oil present may be obtained.

8. *Rape Oil.*—Rape-seed oil, often called also *colza oil*, is obtained from the seeds of various species of *Brassica*, belonging to the general family of the *Cruciferæ*. The refined oil is pale yellow with a distinct odor and an acrid, unpleasant taste. Although it has several times been reported as an adulterant of olive oil it would seem that the latter property would prevent its addition in any quantity to edible oils.

The best quantitative reaction for the detection of rape oil in olive oil is undoubtedly the saponification number. The other values, as specific gravity, refractive index and iodine number, although somewhat higher, do not differ greatly from olive oil. But owing to the large proportion of the glyceride of *erucic acid* present in rape oil its saponification value is low, erucic acid being one of high molecular weight. (See page 159.) The average saponification value of rape oil is about 175, while the average value for olive oil is about 195, and an olive oil having a saponification value below 190 should be regarded as suspicious. Castor oil has also a low saponification value (see page 175) but would be detected by the acetyl value and specific gravity. Rape oil differs also from olive in having a distinctly higher refractive index as compared with the specific gravity.

According to Tolman and Munson,[1] the presence of sulphur compounds in the oils of the *Cruciferæ* furnishes a means for their detection. If the oils are saponified with alcoholic potash and stirred with a silver spoon, the silver will become blackened by the formation of a sulphide. Lewkowitsch however, points out that the test loses much of its value because the cold-drawn rape oils of commerce are free from sulphur, although oils prepared by the extraction process with carbon disulphide may still retain some.

The color reactions for rape oil, including the test with rosani-

[1] *Bur. of Chem., Bull.* **77**, p. 38.

line bisulphite,[1] are unreliable, having failed in the writer's experience to show the presence of rape oil with any certainty in known mixtures.

The same remarks that have been made regarding rape oil apply to mustard-seed oil, which is very similar in its properties, belonging to the same group. Fortunately, however, it is almost never used as an adulterant of olive oil.

Butter

The fat of cows' milk, on account of its universal use, has been selected as a representative edible fat although, as will be shown later, it is not in several respects typical of edible fats in general. Furthermore, the fat is not ordinarily used in a state of considerable purity, as is the case with olive oil, but is always mixed with other constituents from the milk. For this reason the discussion is divided into two parts: the analysis of the butter itself and the examination of the butter-fat.

A.-Analysis of Butter

In the food standards promulgated by the U. S. Department of Agriculture[2] butter is defined as "the clean, non-rancid product made by gathering in any manner the fat of fresh or ripened milk or cream into a mass, which also contains a small portion of the other milk constituents, with or without salt, and contains not less than 82.5 per cent. of milk fat. By acts of Congress approved August 2, 1886, and May 9, 1902, butter may also contain added coloring matter."

General Composition.—As indicated in the foregoing definition, butter consists mainly of the fat of milk, together with a small and varying percentage of water, salt and curd, the latter being made up chiefly of the casein of the milk.

The composition of 351 samples of butter, mostly of English and European origin, is summarized by König[3] in the following table:

[1] Palas: *Ann. chim. anal.*, **1896**, 434.

[2] Office of the Secretary, Circ. **19**.

[3] Chemie der menschlichen Nahrungs- und Genussmittel, 4th Ed., Vol. I, p. 309.

Composition of Butter

	Water, per cent.	Fat, per cent.	Casein, per cent.	Lactose, per cent.	Ash, per cent.
Maximum	35.12	90.92	4.78	1.63	15.08
Minimum	4.15	69.96	0.19	0.05	0.02
Average	13.45	83.70	0.76	0.50	1.59

The extreme limits given in the above table would, however, be rarely met in ordinary practice. The usual grades of marketable butter will generally show: Water, 9.5 to 14.0 per cent.; fat, 82.5 to 88 per cent.; curd, 0.5 to 1.50 per cent.; ash, 0.50 to 5.0 per cent.; the higher ash figures being obtained in the case of salted butter.

Forms of Adulteration.—Apart from the question of the substitution of some foreign fat for the whole or a portion of the butter fat, which will be discussed later, the adulterations practised consist of the inclusion of an excessive amount of water, a deficiency in fat content or the addition of milk or milk powder. Special preparations are on the market for the purpose of enabling the incorporation into the butter of abnormal quantities of water or of milk. The addition of artificial color, being allowed by special Act of Congress, does not constitute an adulteration, although the use of color in oleomargarine and other butter substitutes, since it enables the fraudulent sale of these as butter, is restricted. Preservatives are occasionally added.

Methods of Analysis

Preparation of the Sample.—If the sample for analysis is to be taken from a considerable quantity of butter, great care must be employed in sampling, because the butter is usually not homogeneous in composition and cannot be mixed by stirring. The best plan is to take a fairly large sample of 100 to 200 grams or more, melt it at a temperature of not over 50°C. in a jar or wide-mouthed glass-stoppered bottle and mix by violent shaking. Then cool until sufficiently solid to prevent the separation of the fat and water, taking especial care to shake the sample thoroughly during the cooling.

Water.[1]—Place 1.5 to 2.5 grams of butter in a dish with a flat bottom having a surface of at least 20 sq. cm. and dry to constant weight at the temperature of boiling water, weighings to be made at the end of each hour.

Notes.—If preferred, the butter can be dried on clean dry sand or asbestos, and if a round-bottomed dish has to be used some such absorbent material is necessary.

It is essential if the butter is dried directly that it should be in the form of a very thin layer, since otherwise the water will be covered by the lighter melted fat and evaporation be prevented.

Rapid methods for the determination of water in butter, suitable for dairymen, or for the routine examination of large numbers of samples, have been devised by Patrick[2] and Gray.[3]

Fat.—If no absorbent material was used in the water determination dissolve the dried butter in the dish by treating it with petroleum ether or anhydrous ethyl ether. Transfer the contents of the dish to a weighed Gooch crucible containing ignited asbestos and wash the dish and crucible with ether from a wash bottle until free from fat. Dry the crucible and contents to constant weight at the temperature of boiling water. The increase in weight of the crucible subtracted from the weight of the dried butter gives the weight of fat.

Note.—If the determination of water was made with the help of an absorbent it is evident that the above method will not apply. In this case extract the dried residue from the water determination with several successive portions of petroleum ether, decanting carefully each time through a small filter or Gooch crucible. Collect the filtrate in a weighed flask, evaporate the ether and dry the fat to constant weight in the water-oven, weighing at intervals of an hour. Prolonged heating should be avoided on account of the danger of oxidizing the fat.

Casein or Curd.—Ignite cautiously the crucible containing the residue from the fat determination until the residue is white or nearly so and the weight constant. The loss in weight is the curd. If a more exact determination of the casein is desired

[1] *Bur. of Chem., Bull.* **107,** p. 124.
[2] *J. Am. Chem. Soc.,* **1907,** 1126.
[3] *Bur. of Animal Ind., Circ.* **100.**

the nitrogen may be determined by the Kjeldahl process (see page 25) and the casein calculated by the factor 6.38.[1]

Ash.—The residue remaining in the ignited crucible after the curd has been burned is the mineral matter or ash.

The foregoing determinations may be carried out somewhat more expeditiously on one weighed sample as follows:

Weigh about 2 grams of butter into a Gooch crucible, half-filled with ignited fibrous asbestos, and dry it at 100°C. to constant weight. The loss in weight is the amount of *water*. Then treat the crucible repeatedly with small portions of petroleum ether, using gentle suction, and again dry it to constant weight. The difference between this and the preceding weight will be the amount of *fat*. Now carefully heat the crucible over a small flame or in a muffle until a light grayish ash is obtained. The loss in weight is the amount of *curd*, and the residual increase in weight over that of the crucible and asbestos is the *ash*. If desired, the *salt* may be washed out of the ash and determined by titration with silver nitrate after neutralizing the solution with calcium carbonate.

Salt.—Weigh 10 grams of butter in a small beaker, add 30 cc. of hot water, and when the fat is completely melted transfer the whole to a separatory funnel. Shake the mixture thoroughly, allow the fat to rise to the top, and draw off the water, taking care that none of the fat-globules pass the stopcock. Repeat the operation five times, using 30 cc. of water each time. Make the washings up to 250 cc., mix thoroughly, and titrate 25 cc. in a 6-in. porcelain dish, using $\frac{N}{20}$ silver nitrate with potassium chromate as an indicator.

Preservatives.—*Preparation of the Solution.*[2]—About 50 grams of butter are mixed with 25 cc. of chloroform in a separatory funnel, 100 cc. of dilute (0.1 per cent.) sodium carbonate solution added and the whole mixed, avoiding violent shaking. After the separation of the layers, which may be facilitated by the use of the centrifuge described on page 29, the aqueous layer is examined for preservatives. The principal preservatives to be sought are borax or boric acid, salicylic acid, sodium benzoate

[1] See Allen's Com. Org. Anal., 4th Ed., Vol. II, p. 307.

[2] Richmond and Harrison: *Analyst*, **1902**, 179; **1907**, 144.

and possibly fluorides. Use aliquot portions of the alkaline liquid and follow the methods described in the chapter on Preservatives, pages 90 to 103. The use of dilute sodium carbonate is to ensure the extraction of any free boric acid from the chloroform solution of the fat.

Artificial Colors.—The detection or identification of foreign colors in butter does not have the importance that it does in other foods since the addition of color, being permitted by special legislation, does not constitute an adulteration. If, however, it is desired to learn by comparatively simple tests the character of the color present in a given sample, the two following tests will afford considerable information.

Martin's Test.[1]—Shake about 5 grams of the butter with 25 cc. of a mixture of two parts of carbon bisulphide and fifteen parts of alcohol. After standing for a few minutes the mixture will separate into two layers, the lower consisting of the carbon bisulphide solution of the fat, the upper being the alcoholic solution of the coloring matter. The alcohol layer can be separated and the color tested further by dyeing on threads of wool and silk as described on page 60. Annatto may be detected by the green-blue color produced when the alcoholic solution is evaporated and the residue moistened with a drop of concentrated sulphuric acid.

Notes.—By comparing the color of the alcoholic solution with a known solution of potassium bichromate as a standard it has been found possible to extend the usefulness of Martin's test to the detection of vegetable colors, such as palm oil, in oleomargarine.

Annatto may also be detected in butter by washing the melted fat with hot dilute sodium hydroxide and examining the alkaline solution by the method described under Milk on page 127.

Cornelison's Method.[2]—Melt about 10 grams of the clear, dry fat, and shake well in a separatory funnel with 10 to 20 grams of glacial (99.5 per cent.) acetic acid. If the fat is too hot it will dissolve in the acid but if the test is made at about 35°C. the two layers will separate quickly. Draw off the clear acid, and divide into three portions. To one add a few drops of nitric

[1] *Analyst*, **1887**, 70.

[2] *J. Am. Chem. Soc.*, **1908**, 1478.

acid (sp. gr. 1.42) and to another a few drops of concentrated sulphuric acid, leaving the third portion for comparison.

Uncolored butter in this test leaves the acetic acid colorless and no change is produced by nitric or sulphuric acids. With annatto, turmeric and carrot extract the acetic acid is colored yellow, which remains unchanged with nitric acid but is gradually changed to pink with sulphuric acid. With the coal-tar colors ordinarily used a pink color is given to the acetic acid either before or after the addition of nitric acid.

If sufficient coal-tar color be present it can be removed by the treatment with acetic acid, then extracted from the strongly acid solution by amyl alcohol and tested according to the scheme on pages 67 to 71.

With oleomargarine and the various butter substitutes the question of artificial color is of more importance. Since the use of color enables the product to be sold more readily in substitution for butter the addition of color is usually forbidden. In the United States under the Internal Revenue regulations a prohibitive tax of ten cents a pound is put upon oleomargarine colored in imitation of butter. Of recent years the color most frequently employed has been palm oil, so-called "butter oils" sold for the purpose, consisting of cotton seed oil to which 2 to 5 per cent. of the highly colored palm oil has been added.

The addition of such color will be shown in Martin's test and special methods have also been proposed to detect it.[1] Since, however, these tests must be carried out with absolute attention to the details of the procedure and require considerable experience with commercial oleomargarine in order to interpret the results properly, reference should be made to the original papers.

Interpretation of Results.—Apart from the presence of preservatives, the most common form of adulteration of butter itself is in the incorporation of too large a proportion of water. This may be due to carelessness in making, too high a temperature in churning being a common cause, or to deliberate intent. Butter properly made should not contain more than 12 to 14 per cent. of water at the most, and above 16 per cent. usually constitutes grounds for prosecution for adulteration.

[1] Crampton and Simons: *J. Am. Chem. Soc.*, **1905**, 270; Leach: Food Analysis, 3d Ed., p. 542.

The determination of the amount of curd may be of value in showing the addition of condensed milk or dried milk powder. If more than 1.0 per cent. of curd is found it is probable that some milk product has been added and confirmatory evidence will usually be found in the presence of an excess of water and of milk sugar.

The amount of salt has little significance from the standpoint of detecting adulteration since the quantity added is dependent upon the taste of the consumer.

B.-Examination of Butter Fat

Composition.—Butter fat differs markedly from other animal fats in the number of triglycerides that it contains, more than in any other fat, and in the relatively large proportion of the glycerides of acids of low molecular weight that is present.

The percentage composition, as determined by Browne,[1] employing methods based chiefly upon the solubilities of the lower acids in water of different temperatures, is given below:

Acid	Per cent. of acid	Per cent. of triglyceride
Dihydroxystearic	1.00	1.04
Oleic	32.50	33.95
Stearic	1.83	1.91
Palmitic	38.61	40.51
Myristic	9.89	10.44
Lauric	2.57	2.73
Capric	0.32	0.34
Caprylic	0.49	0.53
Caproic	2.09	2.32
Butyric	5.45	6.23
Total	94.75	100.00

Other observers have found a somewhat different proportion of the various acids, as well as traces of arachidic acid and evidence of the presence of mixed glycerides, but from the standpoint of the food analyst the essential consideration is that the extremely high proportion of the glycerides of soluble and volatile

[1] *J. Am. Chem. Soc.*, **1899**, 807.

fatty acids is characteristic of butter fat, and differentiates it from all others.

Forms of Adulteration.—Practically the only form of adulteration to be looked for in butter fat is the substitution of foreign fats. These may be substituted entirely, as in the case of oleomargarine, in which case the detection of the adulteration does not present any great difficulty, or they may be added in comparatively small amounts, even so little as 10 per cent. In the latter instance, on account of the great variation in composition of genuine butter, due to different conditions under which the milk is produced and the butter manufactured, the problem is by no means an easy one.

The fats commonly employed are lard, beef fat, cotton seed oil and cocoanut oil. These are not so often used singly but combined in various mixtures, which adds to the task of the analyst. Oleomargarine, for example, usually contains beef olein and lard, together with smaller amounts of cotton seed, peanut or palm oils. Cocoanut oil, because of its relatively high content of soluble fatty acids, has been largely used in Europe as an ingredient of "butter oils." Such products, containing oleomargarine, cotton seed oil and cocoanut oil, can be added in considerable quantities to butter fat without affecting most of the quantitative "constants" ordinarily determined.

Artificial preparations, containing tributyrin or triacetin in alcoholic solution, are even added to make up for the decrease in volatile fatty acids caused by the use of animal fats.[1] The introduction in recent years of so-called "hardened oils" produced by the hydrogenation of vegetable oils brings a new possible method of adulteration to the consideration of the food analyst.

The substitution of renovated or "process" butter, although in a strict sense not the substitution of a foreign fat, is best taken up in connection with the detection of oleomargarine, hence is considered here rather than under the analysis of butter itself.

The raw material for the manufacture of renovated butter consists of butter which cannot be sold as such either because of deterioration through rancidity or molding or because, through carelessness on the part of the makers, it possesses an unattract-

[1] Lewkowitsch: Oils, Fats and Waxes, 4th Ed., Vol. II, p. 680.

ive appearance or flavor. The chief recruiting-ground for this material is the country grocery store. The fat, separated from the curd by melting and settling, is aerated to remove disagreeable odors and leave it nearly neutral. This is then emulsified with fresh milk which has been inoculated with a bacterial culture, and the whole is chilled, granulated and churned. The butter is then worked and packed for market in the usual manner. The character of the product has much improved since the early days of the industry, the best grades now approximating the lower grades of creamery butter.

Methods of Analysis

Analytical methods common to fats and oils in general have already been described on pages 151 to 176. The application of these to butter fat and the use of several which possess special value for the examination of butter fat will be considered here. Owing to the presence in butter fat of the lower members of the acetic series of fatty acids, the methods of greatest utility are in general those which afford a measure of (*a*) the mean molecular weight of the fatty acids; (*b*) the mean molecular weight of the soluble and insoluble acids; (*c*) the relative proportion of volatile and fixed fatty acids.

Preparation of the Sample.—Melt a considerable quantity, 50 to 100 grams, of the butter in a beaker on the water-bath, taking care that the temperature does not exceed 60°C. After about 15 or 20 minutes the water, salt and curd will have settled to the bottom, leaving the clear fat on top.[1] A quicker separation may be obtained by centrifuging the melted sample. Decant the layer of fat carefully through a filter paper or loose plug of cotton placed in a warm funnel. The filtering should be done in a warm place to prevent solidifying the melted fat, and the clear filtered sample should be preserved in a stoppered weighing beaker in the ice box until the analysis is completed.

Specific Gravity.—Determine this at $\frac{40°C.}{40°C.}$ following the method described on page 152.

[1] See also page 218 in this connection.

Notes.—The Federal standard for butter fat requires that it shall have a specific gravity of not less than 0.905 at $\frac{40°}{40°}$. The specific gravities of butter fat and some common adulterants determined at practically that temperature as tabulated by Lewkowitsch[1] are given below:

Fat	Specific gravity at 37.8°C. / 37.8°C.
Butter (838 samples)	0.9100–0.9200
Mutton suet	0.9028
Beef suet	0.9037
Oleomargarine	0.9013–0.9038
Cocoanut oil	0.9100–0.9167
Lard	0.905 –0.907

It is evident from the above figures that mixtures of cocoanut oil, for instance, with animal fats could be added in considerable quantities to butter fat, without being detected by the change in specific gravity. As a matter of fact, the specific gravity determination would hardly be expected to yield information of much practical value. Although, as was pointed out on page 148, the fatty acids of low molecular weight have distinctly higher specific gravities, these acids are present in slight amount as compared with the proportion of insoluble fatty acids.

Melting Point.—Either of the methods described on pages 154 and 155, preferably the latter, may be used. Most of the fats used to adulterate butter fat have slightly higher melting points, but the test does not by any means afford as much information regarding adulteration as does the determination of refractive index, which may be carried out in a fraction of the time.

Refractive Index.—Follow the method outlined on page 153, making the determination at about 35°C. and calculating the results to either 25° or 40°C.

Notes.—The refractive index for butter at 25°C. is 1.4590-1.4620; for oleomargarine 1.4650–1.4700. At 40°C. the corresponding values are: 1.4530–1.4560 and 1.4590–1.4640.

As stated on page 154, the refractive index, on account of the ease and rapidity with which the determination can be carried

[1] Oils, Fats and Waxes, 4th Ed., Vol. II, p. 714.

out, has been largely used as a preliminary or sorting test to separate out the suspicious samples of fat or oil for more thorough examination. In the case of a complex fat like butter, however, the assumption must not be hastily made that a normal value for the refractive index necessarily implies the purity of the sample. It is comparatively easy to prepare a mixture of fats and oils which may be added in relatively large quantities to butter and leave the refractive index normal, being detected only by a more thorough analysis. In this instance, as in others, cocoanut oil is of great service to the adulterator, it having a lower index than butter fat, while beef fat and lard are higher.

Reichert-Meissl Number.—The Reichert-Meissl number is *the number of cubic centimeters of tenth-normal alkali required to neutralize the soluble volatile fatty acids distilled from 5 grams of fat.*

Process.—Weigh 5 grams (5.6–5.8 cc.) of the clear filtered fat into a 250 cc. round-bottomed flask, weighing to the nearest centigram only. Add 2 cc. of strong potassium hydroxide solution (1:1) and 10 cc. of 95 per cent. alcohol. Connect the flask with a return-flow condenser and heat on a water-bath so that the alcohol boils vigorously for 25 minutes. At the end of this time, disconnect the flask and evaporate off the alcohol on a boiling water-bath. After the complete removal of the alcohol, add 140 cc. of recently boiled distilled water which has been cooled to about 50°C. The water should be added *slowly*, a few cubic centimeters at a time. Warm the flask on the water-bath until a clear solution of the soap is obtained. Cool the solution to about 60°C. and add 8 cc. of sulphuric acid (1:4) to set free the fatty acids. Drop two bits of pumice, about the size of a pea, into the flask, close it by a well-fitting cork, which is tied in with twine, and immerse it in boiling water until the fatty acids have melted to an oily layer floating on the top of the liquid. Cool the flask to about 60°, remove the cork, and immediately attach the flask to the condenser.

Distil 110 cc. into a graduated flask in as nearly 30 minutes as possible. Thoroughly mix the distillate, pour the whole of it through a dry filter, and titrate 100 c.c. of the mixed filtrate with $\frac{N}{10}$ sodium hydroxide, using phenolphthalein as an indicator.

Multiply the number of cubic centimeters of alkali used by eleven-tenths, and correct the reading also for any weight of fat greater or less than 5 grams.

For example, if 5.3 grams of butter-fat used, and 100 cc. of the distillate require 27.4 cc. of $\frac{N}{10}$ NaOH, 110 cc. would require $27.4 \times \frac{11}{10} = 30.14$ cc. Then $5.3 : 30.14 = 5 : x$; $x = 28.4$. x is the Reichert-Meissl number.

Notes.—Care should be taken that pure reagents are employed. Potassium hydroxide nearly free from carbonate should be used and the solution protected from the carbon dioxide of the air. The alcohol should be free from acid and aldehyde, and in critical work a blank test should be made on the reagents.

The process as described does not recover all the volatile fatty acids, 10 per cent. or more still remaining in the distillation flask. By working under constant conditions, however, a fairly definite portion, and hence comparable results, are obtained. This emphasizes the fact that the method must be strictly followed in its details. Even minor points, such as the weight of fat taken, have a distinct bearing on the result. For example, a butter with a Reichert-Meissl number of 27.6 gave 32.7 when 1 gram was used, and 25.2 when 10 grams were used for the determination. The official Reichert-Wollny method of the British Society of Public Analysts for use in prosecutions under the Margarine Act even specifies the exact dimensions of the apparatus that must be employed.[1] (See also page 208.)

The Reichert-Meissl method is by far the most delicate for showing the presence of animal fats in butter because the differences obtained by it are so much greater than with other methods. This is illustrated by the table given on page 206 in which several common determinations are recorded for butter and for beef fat.

As Lewkowitsch points out, the determination is of paramount importance because a mixture of fats cannot be made that shall have a Reichert-Meissl number normal for butter without using large quantities of butter fat itself in the mixture. On the other

[1] Lewkowitsch: Oils, Fats and Waves, 5th Ed., Vol. I, p. 419; *Analyst*, 1900, 309.

hand, mixtures of cocoanut oil and beef fat can be made of the same refractive index, saponification value, specific gravity or iodine number as genuine butter fat, so that if any one of these were applied as a preliminary or "sorting out" test any proportion of the mixture could be added to butter fat and the sample passed as genuine.

Determination	Beef fat	Butter
Specific gravity	0.945	0.935
Melting point	43°C.	30°C.
Saponification value	196	227
Iodine number	40	35
Insoluble fatty acids	95.5	87.5
Reichert-Meissl number	0.3	28.8

The Reichert-Meissl number for genuine butter varies considerably with the time of year, the food of the cow and the period of lactation, so that it covers quite a range, values having been reported as low as 12 and as high as 40. Cocoanut oil has a Reichert-Meissl value of 6–8, while practically all other edible fats have a value less than 1.0.

Leffman and Beam Method.—The above method may be considerably shortened and practically identical results obtained by carrying out the saponification with glycerin and caustic soda as recommended by Leffman and Beam.[1] The method is as follows:

Weigh 5 grams of the fat into a 250 cc. round-bottomed flask and add 20 cc. of the glycerin-soda solution.[2] Hold the flask with tongs, and heat it directly over a flame until the foaming ceases, and the mixture becomes perfectly clear, which ordinarily requires about 5 minutes. Add to the clear soap solution 135 cc. of water, adding it at first in very small portions to prevent foaming. Finally add the pumice, and sulphuric acid as in the Reichert-Meissl method, and distil without previous melting of the fatty acids.

[1] *Analyst*, **1891**, 153.

[2] Twenty cubic centimeters of 50 per cent. caustic soda solution to 180 cc. of glycerin.

Polenske Number.[1]—The Polenske number is *the number of cubic centimeters of tenth-normal alkali required to neutralize the insoluble volatile fatty acids distilled from 5 grams of fat.*

The principle on which the method is based is that the volatile acids of butter fat, consisting mainly of butyric acid, are largely soluble in water, a small portion only being insoluble. On the other hand, the volatile acids of cocoanut oil, for the detection of which the method is principally used, are largely insoluble. By titrating these two portions separately the difference between butter fat and cocoanut oil can be more readily made apparent.

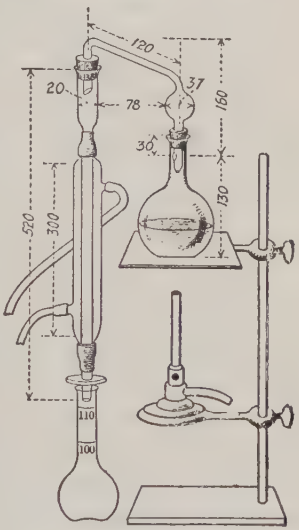


FIG. 44.—POLENSKE apparatus.

Method.—Weigh 5 grams of the filtered fat into a round-bottomed flask of about 300 cc. capacity and saponify according to the Leffman-Beam method (page 206). When saponification is complete allow the mixture to cool slightly and add gradually 90 cc. of recently boiled distilled water. When the soap is all dissolved add 50 cc. of dilute sulphuric acid (25 cc. of concentrated sulphuric acid per liter) and 0.2–0.3 gram of coarsely powdered pumice (grains about 1 mm. in diameter). Connect the flask at once with the condenser, using an apparatus of the form and dimensions shown in Fig. 44, and heat with a small flame, without distilling, until the fatty acids are completely melted. Increase the flame and distil 110 cc. in 19 to 21 minutes, heating the flask through the central opening in the asbestos board. When 110 cc. have been distilled, remove the flame and place a 25-cc. cylinder under the end of the condenser to catch any drops. Mix the distillate and filter through a small dry filter fitting closely to the funnel. One hundred cubic centimeters of the filtrate may be titrated with tenth-normal alkali and phenolphthalein for the Reichert-Meissl number if desired.

[1] Polenske: *Arb. a. d. Kaiserl. Ges.-Amte.*, **1904**, 545; *Z. Nahr. Genussm.*, **1904**, 270.

After filtering the distillate wash the condenser, the 25-cc. cylinder and the 110-cc. flask with three successive portions of 15 cc. each of cold water, pouring each portion also through the filter used to filter the distillate, then rejecting it. Then wash in the same way the condenser, cylinder, flask and filter with three successive portions of 15 cc. each of neutral 90 per cent. alcohol. Titrate the combined alcohol washings with tenth-normal alkali and phenolphthalein. A blank determination should be carried out from the beginning in exactly the same way. The number of cubic centimeters of tenth-normal alkali used, less the number required for the blank, is the *Polenske number*.

Notes.—The soap solution obtained should be perfectly clear and either colorless or faintly yellow. Old or rancid fats, which yield a brown soap solution, are not suited for the Polenske determination.

The method has been studied by a number of observers since it was proposed by Polenske and its value has been shown, but it is essential that the details of the process should be strictly followed and that an apparatus of the form and dimensions shown in the figure be used. Important points are the size of the flask and the length of the condenser, since by increasing these varying results are obtained. The pumice should be of the fineness stated and should be weighed out. The time of distillation should be kept as nearly as possible to that stated, and the flame should be so placed that only the bottom of the flask and not the asbestos support is heated. In order to test the apparatus as employed it may be advisable to carry out a determination on lard, which has a Polenske value of 0.5.

The method is perhaps most useful for the detection of cocoanut oil, for which it was originally devised, but is described at this point because, as suggested above, it can be readily carried out in connection with the determination of the Reichert-Meissl number, the Polenske apparatus serving for both determinations. Other methods, similar in principle but of more limited scope, are described under the detection of cocoanut oil (page 219). For the relative values to be expected in butter and its adulterants see under cocoanut oil, page 219.

Insoluble Fatty Acids.—(Hehner Number).—The Hehner number is *the percentage of insoluble fatty acids obtained from a fat or oil.*

Method.—Weigh out 2.0 to 3.0 grams of the oil or melted fat into a 500-cc. beaker, add 1 cc. of potassium hydroxide solution (1:1) and 20 cc. of 95 per cent. alcohol. Cover the beaker with a watch-glass and heat it on the water-bath until the liquid is clear and homogeneous. Evaporate off the alcohol on the water-bath and dissolve the soap in about 400 cc. of warm distilled water. When the soap is completely dissolved, add 10 cc. of hydrochloric acid (sp. gr. 1.12), and heat the beaker in the water-bath almost to boiling until the clear oil floats. Meanwhile, dry and weigh a thick filter in a small covered beaker. Allow the solution to cool until the fat forms a solid cake on top; filter the clear liquid and finally bring the solid fats upon the weighed filter. Wash the beaker and fat thoroughly with cold water, then wash out the fat adhering to the beaker with boiling water, which is poured through the filter, taking care that the filter is never more than two-thirds full. If the filter paper is of good texture and thoroughly wet beforehand, it will retain the fatty acids completely. If, however, oily particles are noticed in the filtrate, cool it by adding pieces of ice, remove the solidified particles with a glass rod and transfer them to the filter. Cool the funnel by plunging it into cold water, remove the filter, place it in a weighing-beaker and dry it at 100° to constant weight. The fat should be heated about an hour at first, then for periods of about 30 minutes, until the weight is constant within 2 mgs.

Notes.—The residue remaining in the determination of saponification value (see below) may be used to estimate the insoluble fatty acids, if desired, by evaporating off the alcohol, dissolving the soap in water and proceeding as described above.

There is some danger that small quantities of insoluble acids may be washed through the filter and thus the results be too low; on the other hand, with butters containing unusually large amounts of lauric acid, this, being difficultly soluble in hot water, may not be entirely washed out, giving some what higher results.

The percentage of insoluble acids, which includes the small

amount of unsaponifiable matter, varies from 86.5 to 88 in butter fat; other fats and oils give values between 94.5 and 96.

Saponification Number.—Determine the saponification number as described on page 157.

Notes.—The average value for butter fat is 227, which is distinctly higher than for any other edible fat or oil with the exception of cocoanut oil and some samples of palm nut oil. Lard and oleo-products used in the preparation of butter substitutes have lower values than butter, but it is evident that mixtures of these with cocoanut oil can be prepared which will have the correct value for butter.

The determination is of greatest value in the analysis of butter fat when taken in connection with the Reichert-Meissl number. A high saponification value together with a low Reichert-Meissl value would be indicative of adulteration with cocoanut oil. (See page 223.)

Barium Value.[1]—The relation between the mean molecular weights of the soluble and insoluble fatty acids is one of the most valuable means of detecting modern forms of adulteration of butter fat with cocoanut oil and mixtures of similar fats, but the methods for actually separating the individual acids are difficult and tedious. The relation between the Reichert-Meissl and Polenske values answers well for the soluble and insoluble constituents of the volatile acids, and somewhat similar information may be gained regarding the total fatty acids by separating them as their soluble and insoluble barium salts.

Method.—Weigh out 5 grams of the filtered fat and saponify with 50 cc. of $\frac{N}{2}$ alcoholic potash as described under the determination of the saponification number on page 158, making a blank determination as usual, in order to calculate the saponification value. After titrating with the standard acid remove the alcohol as thoroughly as possible by boiling and blowing air into the flask. Dissolve the soap in hot water, cool to about 40°C., and make up to 250 cc. at that temperature. Pipette off 100 cc. at the same temperature[2] into another 250-cc. flask, add 50 to

[1] Avé-Lallemant: *Z. Nahr. Genussm.*, **1907**, 317; Fritsche: *ibid.*, **1907**, 329; Revis and Bolton: Allen's Com. Org. Anal., 4th Ed., Vol. II, p. 288.

[2] The regular laboratory apparatus may be used, since by having the temperature the same in both measurements no error is introduced.

75 cc. of water, allow to stand on the water-bath 5 minutes and add 50 cc. (measured exactly) of barium chloride solution (25 grams crystallized barium chloride per liter). Allow the flask to remain on the water-bath 15 minutes in order to coagulate the insoluble barium salts. Cool, make up to 250 cc. with water and filter off 200 cc. into a beaker. Heat this nearly to boiling, add 1 cc. of hydrochloric acid and 10 cc. of approximately normal sulphuric acid. Filter the barium sulphate on a Gooch crucible, wash free from chlorides with water and finally with two 10-cc. portions of alcohol to remove any fatty acids still remaining. Dry at 100°C. to constant weight. Carry out a blank determination in the same way in order to determine the strength of the barium chloride solution.

Multiply the milligrams of barium sulphate found by 5/4 (to correct for the dilution) and calculate its equivalent in barium oxide. Subtract this from the barium oxide value of the barium chloride used, as determined in the blank. The difference is the barium oxide value of the fatty acids which form insoluble barium salts, and divided by 2, in order to calculate it to 1 gram of fat, gives the *insoluble barium value* (*b*).

The saponification number, calculated to barium oxide, is the *total barium value* (*a*), from which $a - b$ = the *soluble barium value* (*c*). The most characteristic form of expressing the results, as shown in the Notes, is to calculate the numerical value for $b - (200 + c)$.

Example.—Five grams of butter fat require 40.60 cc. of $\frac{N}{2}$ potash for saponification. The weight of barium sulphate obtained as above was 0.3347 gram and the blank was 0.9550 gram. Required the barium value.

$$\frac{40.60 \times 28.05}{5} = 227.8 = \text{saponification number.}$$

$$227.8 \times 1.367 = 311.4 = a.$$

$$955 \times \frac{5}{4} \times 0.6571 = 785 \text{ mg. BaO from blank.}$$

$$334.7 \times 5/4 \times 0.6571 = \frac{275}{510} \text{ mg. BaO in excess.}$$

$$\frac{510}{2} = 255 \text{ mg. BaO in insoluble acids} = b.$$

$$a - b = 311.4 - 255 = 56.4 = c.$$

$$b - (200 + c) = -1.4.$$

Notes.—The value of the expression $b - (200 + c)$ varies considerably with pure butter fat but is almost invariably *negative*, while for the other edible oils and fats it is always *positive* and not less than + 39.0. The addition of 10 per cent. of cocoanut oil or beef fat to butter raises it to a positive value. One exceptional advantage of the method is that the effect of the simultaneous presence of lard and cocoanut oil is additive and not mutually destructive as in some other methods.

Revis and Bolton[1] state that they have observed a few instances of mixtures of cocoanut oil and butter in which negative values were obtained for $b - (200 + c)$, but in such mixtures they have always found (b) to exceed 260.0, while in genuine butter fat (b) is always well below 260.0.

The following table, taken from their work, illustrates clearly the value of the method.

	R. M. No.	Pol. No.	Sapon. No.	Total BaO (a)	Insol. BaO (b)	Sol. BaO (c)	$b-(200+c)$
Butter *A*	28.7	3.2	228.4	312.2	255.4	56.8	− 1.4
Butter *A* + 10 per cent. cocoanut oil	26.6	4.1	231.1	315.9	262.8	53.1	+ 9.7
Butter *C*	30.5	3.5	227.0	310.3	255.1	55.2	− 0.1
Butter *C* + 10 per cent. cocoanut oil	28.0	4.3	230.5	315.1	263.6	51.5	+12.1
Butter *D*	30.8	2.9	224.8	307.3	252.8	54.5	− 1.7
Butter *D* + 10 per cent. lard	27.7	2.4	221.8	303.2	254.6	48.6	+ 6.6

Other methods for detecting cocoanut oil are discussed on page 219.

Detection of Synthetic Triacetin.[2]—About 30 grams of the butter fat, whose Reichert-Meissl value has been determined, are placed in a 600-cc. flask with 150 cc. of water and 150 cc. of 95 per cent. alcohol. Some coarsely powdered pumice is added and the mixture boiled gently under a reflux condenser for an hour. After cooling, the fat is separated from the alcoholic layer and heated on the water-bath until all water and alcohol are evaporated. On the dry fat the Reichert-Meissl number is again determined. With butter and other natural fats the Reichert-

[1] *Loc. cit.*

[2] Fincke: *Z. Nahr. Genussm.*, **1908**, 666.

Meissl value remains practically unaltered, while in the presence of triacetin the second result is distinctly lower than the first.

Note.—The method is based on the solubility of triacetin in dilute alcohol. Mixtures of 4–6 per cent. of triacetin and similar synthetic preparations with lard have been used to adulterate butter, since the Reichert-Meissl number of the mixture, being about 29, enables it to escape detection by the ordinary tests. The presence of 2 per cent. of triacetin, according to Fincke, caused the Reichert-Meissl number to fall from 28.35 to 19.52 when the sample was treated as described above.

Interpretation of Results

The detection of foreign fats in butter should be classed among the most difficult problems of food analysis, and requires thorough physical and chemical examination in order to show the adulteration with certainty in all cases. The problem is in some ways a more difficult one than the examination of olive oil for adulterants because of the very great natural variation that occurs in butter fat with manner of feeding, time of year and period of lactation.

An excellent idea of the variations to be expected and the proportion of samples that will fall within the usual limits is obtained from the following tabulation of 357 samples of British butters.[1]

Table XXXI.—Analyses of Butter Fat

No. of samples	Reichert-Wollny number	Sp. gr. 37.8°/37.8°	Saponification number	Soluble acids as butyric, per cent.	Insoluble acids, per cent.	Mean molecular weight of insoluble acids
7	22.5	0.9101	219.6	4.3	90.1	266.9
17	23.5	0.9104	221.4	4.5	89.7	265.5
15	24.5	0.9108	223.2	4.7	89.4	265.0
27	25.5	0.9110	223.4	4.8	89.3	264.2
37	26.5	0.9113	225.4	4.9	88.9	261.9
51	27.5	0.9114	226.7	5.2	88.7	261.7
78	28.8	0.9118	228.3	5.4	88.4	260.9
56	29.5	0.9120	229.9	5.6	88.3	259.6
41	30.5	0.9123	231.4	5.8	87.9	260.1
18	31.3	0.9125	232.3	5.7	87.9	258.0
10	32.6	0.9130	232.6	6.0	87.7	257.8

[1] Thorpe: *J. Chem. Soc.*, **1904**, 254.

In a general way it may be said that the presence of animal fats in any quantity would be shown by the saponification and Reichert-Meissl numbers. Since the saponification numbers of the animal fats are usually below 200 and the Reichert-Meissl numbers less than 1.0, it is evident that the presence of animal fats (lard, beef, etc.) will lower both of these values. Vegetable oils would be shown by an increase in the iodine value, which with butter fat varies from 26 to 38, while olive oil has about 80 and the other fluid oils 100 or more. For conclusive evidence of the presence of a vegetable oil it may be necessary to make the phytosteryl acetate test (page 176), the presence of the oil being proven by a melting point of the acetate of 117°C. (corr.) and above.

Of all the general methods which have been described the Reichert-Meissl is undoubtedly the most useful as well as the most delicate. This is readily seen when it is recollected that in the saponification number the values obtained for butter and for oleomargarine differ by only about 15 per cent. and in the iodine number the value for oleomargarine is only about twice that for butter. With the Reichert-Meissl number, on the other hand, the value is twenty-five times as much in one case as in the other. (See also page 215.)

It should be repeated, however, that no one test will show with certainty the addition of comparatively small amounts of mixtures of vegetable and animal oils as practised at present. Further, laboratories where samples are "sorted out" by a quick preliminary test, as the refractive index, can necessarily detect only the grosser forms of adulteration.

The detection of several specific forms of adulteration is described in more detail below.

Detection of Oleomargarine.—The raw materials used in the manufacture of oleomargarine are "oleo oil," which is mainly the olein of beef fat, neutral lard and cotton seed oil, together with smaller amounts of butter and cream or milk. The natural effect of these upon the constants usually determined for butter fat would be to increase the percentage of insoluble fatty acids and the iodine number, and to lower the Reichert-Meissl number, saponification number and specific gravity.

If the question involved is merely the distinction between

oleomargarine and butter it is comparatively simple, the constants noted above showing distinctly different values, as summarized in the following table.[1]

TABLE XXXII.—CONSTANTS OF BUTTER FAT AND OLEOMARGARINE

	Sp. gr. at 100°C.	Hehner number	Saponification number	Reichert-Meissl number	Refractive index at 35°
Butter fat, maximum....	0.870	89.6	233	34.86	1.4578
Butter fat, minimum....	0.867	85.6	222	22.7	1.4557
Oleomargarine, maximum	0.862	95.5	203	5.5	1.4625
Oleomargarine, minimum	0.858	92.5	192	0.5	1.4613

When the case is so simple a single determination may be all that is needed to show the character of the fat in question. When oleomargarine is added in small amounts, however, the Reichert-Meissl method is the only satisfactory guide. This is well shown in the following figures showing the results obtained with a sample of pure butter and with the same butter mixed with varying proportions of foreign fat, which are taken from the table quoted by Leach.[2]

	Hehner number	Saponification number	Reichert-Meissl number
Pure butter	88.0	224.0	26.0
Butter, 95 per cent.; foreign fat, 5 per cent.	88.35	222.6	24.7
Butter, 90 per cent.; foreign fat, 10 per cent.	88.70	221.2	22.2
Butter, 85 per cent.; foreign fat, 15 per cent.	89.05	219.8	20.9
Butter, 80 per cent.; foreign fat, 20 per cent.	89.40	218.4	19.6
Butter, 75 per cent.; foreign fat, 25 per cent.	89.75	217.0	18.3

There are no specific qualitative tests for oleomargarine, although the taste and odor are characteristic and enable experts to distinguish it from butter. In some European countries it is required that some oil easily detected by chemical tests, as sesame oil, shall be added to oleomargarine. The "Spoon Test" (page 217) will serve to differentiate oleomargarine from

[1] Leach: Food Inspection and Analysis, 3d Ed., p. 544.
[2] *Loc. cit.*

butter, although it should be remembered that practically the same test is given by renovated butter.

Detection of Renovated Butter.—Since the fat of renovated butter is genuine butter fat, it is evident that the determination of the usual "constants" will be of practically no help. Crampton[1] has shown that the changes in composition of butter fat caused by blowing air through it in the manufacture of renovated butter are less than the variations to be expected naturally in the fat itself. The only satisfactory methods for detecting renovated butter are those based on physical differences in the fat, caused by its being melted and then suddenly cooled in the process of manufacture, or on the character of the curd. Of these the most important are given below. There is no test which will show the addition of small quantities of renovated butter to genuine butter.

1. *Microscopic Examination.*—Pure, fresh butter is not ordinarily crystalline in structure. Butter which has been melted, however, and fats which have been liquefied, and allowed to cool slowly, show a distinct crystalline structure, especially by polarized light. If only fresh butter were sold, and all adulterants had been previously melted and slowly cooled, this method would be all that would be necessary for the detection of adulteration. As it is, however, it is most useful in making comparative examinations of samples which have been subjected to the same conditions.

It is, of course, necessary in the case of butter to be certain that it has been kept cool and not allowed to melt previous to making the microscopic examination.

If a bit of the fresh, unmelted sample, about the size of a pinhead, is taken from the center of the mass and pressed out on a slide by gentle pressure on the cover-glass, it ought to show a fairly uniform field when examined with a 16-mm. objective.

In the case of renovated butter, however, there is a distinct difference to be noted in the appearance of the field. With genuine butter the field is much more clear and free from opaque masses of curd than with renovated butter. When the field is examined by reflected light, turning the mirror so as not to pass light through the slide, these opaque masses in the case of reno-

[1] *J. Am. Chem. Soc.*, **1903**, 364.

vated butter show strikingly as white masses against a dark background.

When examined also by polarized light, using a low power objective and a selenite plate, pure butter shows no crystalline structure and an even color, while renovated butter shows a mottled field with variegated colors. The same effect is given by oleomargarine, and for the same cause. The test should be made on samples of genuine and renovated butter likewise in order to become familiar with the distinction before drawing definite conclusions regarding an unknown sample.

2. *"Spoon" or "Foam" Test.*[1]—Melt a piece of the sample as large as a small chestnut in an ordinary tablespoon or a small tin dish. Use a small flame and stir the melting fat with a splinter of wood (such as a match). Then increase the heat so that the fat shall boil briskly, and stir *thoroughly*, not neglecting the outer edges, several times during the boiling.

Oleomargarine and renovated butter boil noisily, usually sputtering like a mixture of grease and water when boiled, and produce little or no foam. Genuine butter usually boils with much less noise and produces an abundance of foam, often rising over the sides of the dish or spoon when the latter is removed temporarily from the flame. The difference in regard to the foam is very marked.

Note also the appearance of the particles of curd after the boiling. With genuine butter these will be very small and finely divided, hardly noticeable in fact, while with oleomargarine and renovated butter the curd gathers in much larger masses or lumps.

Notes.—In genuine butter the curd is somewhat different in composition from that of renovated butter or oleomargarine in that it consists largely of the milk proteins that are insoluble in water, and hence accompany the separated cream. The curd of renovated butter or oleomargarine, on the other hand, comes from the proteins of the milk added directly in the process of manufacture, and consists mainly of coagulated casein. Hence its different appearance and behavior as regards foaming.

The crackling and sputtering of the fat in the case of oleomargarine and renovated butter are due to the fact that in the

[1] *U. S. Dept. Agr., Farmers' Bulletin,* No. 131.

process of manufacture the melted fat is sprayed into ice water, and the cooled particles enclose some water.

Another effect, due to the difference in the curd of butter and of renovated butter, is seen in the appearance of the fat when the sample is melted on the water-bath.[1] The curd of pure butter, being more gelatinous and cohesive, will readily settle out in a few minutes, leaving a fairly clear fat layer. The curd of renovated butter (and to a certain extent of oleomargarine also), being composed of the more granular casein, does not settle so readily but gives to the melted fat a turbid, cloudy appearance, even after standing for several hours.

3. *The Waterhouse Test.*[2]—This test, although proposed originally as a test for distinguishing oleomargarine from butter, is also useful in detecting renovated butter. Crampton[3] finds it the most satisfactory of all the various tests.

Heat about 50 cc. of well-mixed sweet milk nearly to boiling and add 5–10 grams of the sample. Stir the mixture, preferably with a small wooden stick, until the fat is melted. Place the beaker in ice water and when the fat begins to solidify, which usually requires about 10 minutes, stir thoroughly until the fat has hardened. Butter fat mixes with the milk, does not adhere to the wooden rod, and is slow to rise to the surface when the stirring is stopped. Oleomargarine is readily collected by the rod into a sticky lump, while renovated butter gathers in granular masses on the surface when the stirring is interrupted and does not readily form a compact lump.

The test is based on physical differences in the character of the fat which has been previously melted rather than on any difference in chemical constitution, as is shown by the fact that the test can be carried out nearly as well in water as in milk.

In all of the above tests it must be remembered that very similar results are given by oleomargarine, which must be differentiated from renovated butter by the usual chemical or physical tests on the fat. Further, the statement will bear repeating that no definite conclusion should be drawn from these

[1] Hess and Doolittle: *J. Am. Chem. Soc.*, **1900,** 151.

[2] Parsons: *J. Am. Chem. Soc.*, **1901,** 200; Patrick: *Bur. of Chem., Bull.* **67,** p. 115.

[3] *J. Am. Chem. Soc.*, **1903,** 363.

simple tests until the student has become familiar with them by a comparative study of known samples.

Detection of Cocoanut Oil.—The third form of adulteration to be considered is by far the most difficult of detection. The adulterant is one which resembles butter fat in several of its constants more closely than any other fat which is used, and it is commonly added in relatively small amounts and often in mixtures with other fats. Considerable space is devoted to it here because of the difficulty of detecting it and because its use as an adulterant has assumed large proportions in Europe and is increasing in this country.

The fatty acids of cocoanut oil are made up as follows:[1]

Caproic acid	0.55 per cent.
Caprylic acid	6.65 per cent.
Capric acid	11.00 per cent.
Lauric acid	55.40 per cent.
Myristic acid	16.40 per cent.
Oleic acid	10.00 per cent.

Cocoanut oil thus differs from other vegetable oils and from the animal body fats in its high content of volatile fatty acids. It differs from butter fat in the large proportion of these fatty acids insoluble in water, and in the high percentage of caprylic acid. (Compare with the table on page 200.) Upon the former fact is based its detection in butter fat by the Polenske method, and upon the latter its differentiation by various methods depending upon the separation of the acids into different groups according to the solubility of certain of their metallic salts.

(*a*) **Polenske Number.**—This method, the manipulation of which has already been described in connection with the Reichert-Meissl process (page 207), is the oldest and has been most widely used for the detection of cocoanut oil. The results with butter fat vary between 1.5 and 3.0, with palm oil between 8.5 and 11.0, and with cocoanut oil between 16.8 and 17.8. Since the Polenske number varies in butter in a general way with the Reichert-Meissl number, the two should be considered together, as shown in the following table, condensed from numerous results given by Polenske.

[1] Heiduschka and Pfitzenmeier: *Z. Nahr. Genussm.*, **1912**, 31.

TABLE XXXIII.—COMPARISON OF POLENSKE AND REICHERT-MEISSL NUMBERS

Corresponding values		
Reichert-Meissl number	Polenske number	Highest permissible Polenske number
20–21	1.3–1.4	1.9
21–22	1.4–1.5	2.0
22–23	1.5–1.6	2.1
23–24	1.6–1.7	2.2
24–25	1.7–1.8	2.3
25–26	1.8–1.9	2.4
26–27	1.9–2.0	2.5
27–28	2.0–2.2	2.7
28–29	2.2–2.5	3.0
29–30	2.5–3.0	3.5

For example, a butter having a Reichert-Meissl number of 25–26 should show normally a Polenske number of 1.8–1.9 and a value of more than 2.4 indicates the presence of cocoanut oil.

By the addition of 10 per cent. of cocoanut oil the Polenske number is usually increased about 1.0.

(*b*) **Cadmium Number.**[1]—Weigh out 2.5 grams of fat and saponify with 10 cc. of glycerin-soda solution as in the Leffmann-Beam method, page 206. Dissolve the soap in 50 cc. of hot boiled water, cool the solution to 55–60°C. and set free the fatty acids with 25 cc. of dilute sulphuric acid (25 cc. concentrated sulphuric acid per liter). After standing about 10–12 hours filter the solid fatty acids on a filter plate in a small funnel, using very gentle suction if necessary, and wash with 50 cc. of cold water. Transfer the residue of fatty acids, together with the filter paper, to a suitable tube or small flask, washing off any particles of fatty acid which may cling to the funnel with 5 cc. of 1 per cent. sulphuric acid.

Distil in a current of steam so that 200 cc. pass over in 35 to 40 minutes, then in place of the distilling apparatus attach to the condenser a flask containing neutral alcohol and distil

[1] Paal and Amberger: *Z. Nahr. Genussm.*, **1909**, 1;23.

about 50 cc. in order to wash traces of fatty acids from the condenser.

To the 250 cc. of mixed alcohol and water in the distillate, which will be turbid from the suspended insoluble fatty acids, add a few drops of phenolphthalein and carefully neutralize with $\frac{N}{10}$ sodium hydroxide, which will require 10–15 cc. Add 1 cc. in excess and evaporate on the water-bath to about 40 cc., dilute to 50 cc. and neutralize exactly with $\frac{N}{10}$ acid. Add 2 cc. of a 20 per cent. cadmium sulphate solution, stir thoroughly and let stand an hour, then filter through a Gooch crucible, and wash with 50 cc. of water. Dry to constant weight at 102°–106°C. The weight of the precipitate, expressed in milligrams, is the *Cadmium Number*.

Notes.—The method is based on the fact that while the cadmium salts of butyric and caproic acids are readily soluble those of the higher acids are nearly insoluble and hence readily separated from the former. By distilling with steam some lauric and myristic acids are distilled also and consequently the differentiation from butter fat increased.

From numerous tests, Paal and Amberger conclude that the cadmium number of butter lies between 70 and 90, and of cocoanut oil between 441 and 470. An addition of 10 per cent. of cocoanut oil to butter raises the number about 33.

Since the volatility of the fatty acids is somewhat dependent on the apparatus and method of distilling, these values should be regarded as only approximate and the exact values for butter and for cocoanut oil should be worked out for the particular apparatus employed. Paal and Amberger describe a special form of steam distillation flask, quite similar to that used by Hortvet for volatile acids in wine (see page 440), which they recommend for the determination.

Experiments in the writer's laboratory[1] have shown that satisfactory results, which agree well with those of Paal and Amberger for pure butter and various mixtures of butter with cocoanut oil, may be obtained by placing the fatty acids in a rather wide tube which is immersed in a saturated brine bath in

[1] A. A. Cook: Thesis, Mass. Inst. Technology, 1915.

order that the temperature during the steam distillation may be kept at 107–108°C. This facilitates the distillation and prevents the condensation of steam.

(*c*) **Hinks' Microscopic Method.**[1]—Dissolve 5 cc. of the melted and filtered fat in 10 cc. of ether in a test-tube which is then corked and placed in pounded ice for half an hour. At the end of this time some of the solid glycerides will have settled out, leaving a clear ethereal layer. Filter rapidly through a plaited filter, and evaporate the ether (*away from a flame*). Transfer the residual fat to a test-tube and boil it with 3–4 times its volume of 96–97 per cent. alcohol. On allowing the solution to cool to room temperature most of the fat separates. Place the tube in water at 5°C. for 15 minutes, filter the clear alcoholic solution rapidly into another test-tube and place the latter in crushed ice in an ice-box for 2–3 hours. Remove with a glass tube a portion of the flocculent precipitate which forms, place it on a slide, cover loosely with a cover glass and examine it under the microscope, using a 4-mm. objective.

Since the crystals melt at about 5°C., the slide must be kept cold during the microscopical examination, which is readily done by keeping it on a Petri dish containing clear pieces of ice.

Notes.—The deposit in the case of butter will show under the microscope as round granular masses, while with cocoanut oil, clusters of fine needle-like crystals will be observed. With mixtures, both forms will be seen, the cocoanut oil crystals sometimes appearing as separate clusters but more frequently attached to the butter fat granules. Typical fields are shown in Figs. 106 to 108, page 502, from Lewkowitsch's Oils, Fats and Waxes.

It is important that the alcohol should be of the strength stated, since with weaker alcohol there is a tendency for the deposit from butter fat to be more or less crystalline.

Lard and beef fat give crystals somewhat similar to those obtained from cocoanut oil, but if these are present in quantities of 10 per cent. or more, the characteristic granular appearance of the butter fat masses is almost entirely obliterated.

The method requires some practice with the microscope to be carried out successfully, but after experience has been gained by a study of known samples, yields good results, as little as 5 per

[1] Hinks: *Analyst*, **1907**, 160.

cent. of cocoanut oil being detected by careful searching of the microscopic field.

(*d*) **The "Juckenack Difference."**—Since in cocoanut oil the saponification number is higher and the Reichert-Meissl number lower than in butter, the numerical difference between the two is a better indication of the presence of cocoanut oil than either one alone.[1] For butter the value of the expression:

Reichert-Meissl number—(saponification number—200)

lies between + 4.25 and − 3.50, averaging about 0.0. The corresponding value for cocoanut oil varies from − 40 to − 60.

The "barium value" has already been described (page 210) and its value in showing the presence of cocoanut oil pointed out. In cases of extreme doubt final resort may be had to the phytosteryl acetate test (page 174), but this requires considerable experience for satisfactory results.

It has been shown by several observers that if cows are fed with cocoanut cake or the residue from the oil presses, the butter will show practically all the changes in its constants that would be brought about by the addition of cocoanut oil directly. Somewhat similar results are caused by feeding largely with turnips or turnip tops. These practices, however, are not sufficiently common to prove a serious drawback to the analytical methods.

In general, it has been shown by numerous investigators that feeding with materials rich in vegetable oil, especially the press-cake from oil seeds, such as cotton seed, will cause the absorption of a part of the oil into the milk and that the butter prepared from this source will have many of the characteristics of a mixture with the oil in question. This in itself constitutes a strong argument for the phytosteryl acetate test, since careful experiments have shown conclusively that phytosterol is not transferred to either the milk or the body fat as a result of feeding with vegetable oils.

Detection of Hardened Oils.—A possible form of adulteration which in the near future will undoubtedly assume much greater analytical importance is the addition to edible fats, as lard or butter, of the various "hardened" oils. These are made by passing hydrogen through the heated oil in the presence of a suitable catalyzer, as finely divided metallic nickel or palladium. The

[1] Juckenack and Pasternack: *Z. Nahr. Genussm.*, **1904,** 193.

products thus made have as high a degree of edibility as the oils from which they are prepared, and apart from any possible danger from the traces of nickel that may remain in the product, are probably unobjectionable as food material. The keeping qualities of the fat are distinctly improved, samples of hardened oils having been kept for 18 months exposed to damp air without showing any signs of rancidity. Some of the hardened oils have their appearance so changed that they resemble the edible animal fats and can hardly be distinguished from them as regards color, consistency and flavor. For example, medium hard peanut oil is so completely like neutral lard, and hardened whale oil so like mutton tallow that they cannot be distinguished by outward appearance from the genuine fats. Even so odorous a product as fish oil becomes changed into a solid odorless fat, thereby greatly increasing its possibilities as an adulterant.

These changes in the general properties of the oils by hydrogenation are accompanied by changes equally great in the analytical constants, especially those depending on the presence of unsaturated fatty acids. By the absorption of hydrogen such acids as oleic, linoleic and linolenic are more or less completely transformed into stearic acid. In general this means a decrease in the iodine number and refractive index, and an increase in the melting and solidifying points. The saponification number remains practically unchanged. This change is a gradual one and can be stopped at any point desired, as illustrated in the following table[1] which shows the constants of the original oil (cotton seed) and of the hardened product at different stages.

	Original oil	Hardened oil		
Appearance	Clear liquid	Solid particles floating in oil	Soft greasy solid	Brittle solid
Refractive index, 40°C..	1.4644	.....	.....	1.4578
Iodine value of fatty acids	110	94	55	22
Titer test	34.7°C.	37.0°C.	42.5°C.	52.2°C.
Saponification value	197	196	196	192

[1] Knapp: *Analyst*, **1913**, 102.

Such characteristic tests as the hexabromide test for fish oils (page 192) lose their value in the hydrogenated product on account of the change of the highly unsaturated acids to saturated acids, chiefly arachidic.

The specific color reactions are variously affected, the Halphen test for cotton seed oil, for instance, failing to give positive results with the hardened oils, while the Baudouin reaction, on the other hand, is made even sharper by the hydrogen treatment. The studies of Kreis and Roth[1] indicate that of the general color reactions for showing the presence of seed oils in animal fats, only the Bellier test is suitable for use with hardened oils.

This test is as follows: Mix 5 cc. of the melted, filtered fat with 5 cc. of colorless nitric acid (sp. gr. 1.4) and 5 cc. of a cold saturated solution of resorcin in benzol, and shake the mixture violently for 5 seconds in a stout, glass-stoppered tube. If during the shaking or within the next 5 seconds, a red, violet or green color is produced, seed oils are indicated. The later appearance of color should be disregarded. As in the case of other color reactions, experience should be gained by tests on known mixtures.

According to Bömer[2] phytosterol and cholesterol are unchanged by the hydrogenation, the acetyl esters obtained from hardened peanut, sesame and cotton seed oils melting at 126°–129°C., while the corresponding acetate from hardened whale oil had a melting point corresponding to cholesterol. The phytosteryl acetate test (page 174) thus promises to be of great value in showing the presence of hardened vegetable oils in animal fats, its conclusions being more reliable, if the test is made with care, than the usual results of color reactions.

With some of the oils, as the fish oils, in which one of the chief results of the hydrogenation is the production of arachidic acid, the presence of this as shown by the Renard or Bellier test, page 184, may be used as a means of identification.

It has been suggested[3] that tests for the traces of the nickel catalyst which remain in the product will be the best means of

[1] *Z. Nahr. Genussm.*, **1913**, 81.
[2] *Z. Nahr. Genussm.*, **1912**, 104.
[3] Knapp: *Loc. cit.*

showing the presence of hardened oils. The most delicate test for nickel is dimethylglyoxime, $CH_3C(NOH).C(NOH)CH_3$, which according to Fortini[1] is best used in alkaline solution. Fortini's reagent is prepared by mixing 0.5 gram of dimethylglyoxime, 5 cc. of 98 per cent. alcohol, and 5 cc. of concentrated ammonium hydroxide, in the order given. This yields a clear, faintly yellowish liquid which may be kept in glass-stoppered bottles without change. The test is carried out as follows:

Heat 50 grams of the fat in a flask with 20 cc. of hydrochloric acid, with continued vigorous shaking. Allow the mixture to separate while hot and evaporate a portion of the acid solution to dryness in a porcelain dish. Heat the residue for a few moments in an oxidizing flame and add a drop of the above reagent. When nickel is present there will appear in a few seconds a rose color due to the reaction with the nickel oxide present on the surface of the metallic nickel.

This test is of value, of course, only in those cases where nickel rather than platinum or palladium has been used as a catalyzer and, furthermore, Prall[2] has pointed out that a few pure untreated oils may give a red color with dimethylglyoxime.

According to Kerr,[3] the latter difficulty may be avoided by destroying organic matter in the acid extract before testing for nickel.

Ten grams of the fat are heated on the steam bath with 10 cc. of dilute hydrochloric acid (sp. gr. 1.12), with frequent shaking, for 2–3 hours. The fat is then removed by filtering through a wet filter paper, the filtrate being received in a white porcelain dish. The filtrate is evaporated to dryness on the steam bath, 2–3 cc. of concentrated nitric acid being added, after it has been partly evaporated, to insure the destruction of all organic matter. The residue is dissolved in a few cubic centimeters of distilled water and a few drops of a 1 per cent. solution of dimethylglyoxime in alcohol added. A few drops of dilute ammonia are then added. The presence of nickel is shown by the appearance of the red color as above.

[1] *Chem.-Ztg.*, **1912**, 1461.
[2] Bömer: *Loc. cit.*
[3] *J. Ind. Eng. Chem.*, **1914**, 207.

The amount of nickel present may be estimated by comparing the color with that of a standard solution of a nickel salt.

Further information on the changes in analytical constants produced by the hydrogenation of oils will be found in a review of the literature published by Ellis.[1]

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[1] *J. Ind. Eng. Chem.*, **1914**, 117.

CHAPTER VI

CARBOHYDRATE FOODS

General Methods

Principal Carbohydrates.—Although thirty or more sugars have been isolated, those which are of direct importance to the food analyst are comparatively few in number. Important types of all the classes of carbohydrates, however, are among the common ones that we shall consider in this chapter.

They include:

Monosaccharides.—(Hexoses), Dextrose,[1] Levulose, Galactose; (Pentoses), Xylose, Arabinose.

Disaccharides.—Sucrose, Lactose, Maltose.

Polysaccharides.—Starch, Dextrin, Cellulose, Pentosans.

Monosaccharides.—*Dextrose.*—Dextrose.—(*d*-glucose, grape sugar), is of very wide natural distribution, being found as such in the blood of animals and in the juices of plants. It is found, also, in combined form as glucosides, for instance, salicin or amygdalin. The sugar is sweet to the taste and readily soluble in water or hot alcohol. The complex polysaccharides, of which starch and cellulose are typical, may be regarded as anhydride complexes which can be hydrolyzed into dextrose molecules. Most of these, as well as the disaccharides and the trisaccharide raffinose, yield dextrose upon hydrolysis with acids.

In the analysis of food products careful distinction should be made between dextrose or *d*-glucose and *commercial glucose*, which is not a natural product at all, nor even a definite chemical substance, but a variable mixture of dextrose, dextrin and maltose, made by the incomplete hydrolysis of starch (usually corn) with dilute acid. The common confusion of this product with the true glucose warrants the insertion of the following quotation from an authority on the subject:

[1] On account of their simplicity and wide analytical use, the terms *dextrose* and *levulose* are used here instead of the more strictly correct *d*-glucose and *d*-fructose.

"It is remarkable how few of the apparently well-informed know what "*commercial glucose*" really is. This is due to the confusion of terms which associates this misnamed starch product with grape sugar and dextrose. It is quite true that dextrose (glucose) is an ingredient of commercial glucose, but the dextrose in the commercial glucose of to-day is the least important ingredient, both in quantity and for the qualities which it imparts to the product.

"Pure cane molasses is much more entitled to the name "glucose" than is this starch product so far as its composition and characteristics are concerned. Yet encyclopedias, scientific texts and popular writings are full of erroneous statements confusing "glucose" with dextrose and the glucoses. Indeed, its most earnest advocates and advertisers have been responsible for glaring misstatements, both in the public prints and in court testimony, through implication of facts relating to dextrose and the glucose sugar to this well-defined product of quite different characteristics. This might not be so surprising except for the tremendous magnitude and importance which the manufacture of this product has in the world's food economy."[1]

Levulose (*d*-fructose, fruit sugar) is usually present with dextrose in plant juices, very likely as the product of hydrolysis of sucrose by enzyme action, and exists in honey particularly, making up nearly 40 per cent. of that product. Levulose is easily soluble in cold water or in hot alcohol. Its action on polarized light is strongly levo-rotatory, the degree of rotation being much affected by changes in temperature.

Galactose.—This sugar usually exists as a condensation product, *galactan*, a constituent of many gums, mucilages, hemicellulose and other plant tissues. It has considerable analytical importance as a product of hydrolysis of lactose and of the galactans. Small quantities of copper-reducing matters present in some foods and reported as starch from the results of acid hydrolysis are undoubtedly galactan.

Pentoses.—Of the pentoses, or monosaccharides containing five carbon atoms ($C_5H_{10}O_5$), the only ones that need to be mentioned are arabinose and xylose, which are the products of hydrolysis of araban and xylan. (See page 231.)

[1] G. W. Rolfe: Rogers and Aubert's Industrial Chemistry, 2d Ed., p. 772.

The pentoses are characterized by strong reducing action on Fehling's solution and by their being converted into furfural when distilled with hydrochloric acid.

Disaccharides.—*Sucrose.*—On account of its importance as a food product, sucrose is the best known of the sugars. It is found in many plants, in quantities ranging from 0.1 per cent. to 25 per cent. of the fresh material. The chief commercial sources are the juices of the sugar beet, sugar cane and sugar maple. As prepared for consumption, it is one of the purest food products, ordinary granulated sugar being usually about 99.85 per cent. pure sucrose, the remainder being mainly water. The hydrolysis products of one molecule of sucrose are one molecule of dextrose and one of levulose, the mixture being commonly known as *invert sugar.*

Lactose.—As sucrose is a sugar of distinctly vegetable origin, so lactose is derived entirely from the animal kingdom, being present in the milk of mammals in quantities varying ordinarily between 3 and 8 per cent. The sugar usually exists in the form of lactose monohydrate, $C_{12}H_{22}O_{11} + H_2O$, the water of crystallization being lost only when the sugar is heated to 130°C. By treating with acids under proper conditions, lactose is hydrolyzed into equal parts of dextrose and galactose, the hydrolysis being, however, considerably more difficult than with sucrose. Lactose has practically no sweet taste and is much less soluble in water than cane sugar.

Maltose.—This sugar is ordinarily found in foods as the result of the action of amylases or starch-splitting enzymes such as occur in germinated barley or malt. It is found also as an intermediate product in the hydrolysis of starch by acids. Maltose is distinguished from the simple reducing sugars by its failure to reduce cupric acetate solution. When hydrolyzed by acids, one molecule of maltose yields two molecules of dextrose.

Polysaccharides.—*Starch.*—$(C_6H_{10}O_5)$ is the most important of the polysaccharides, being stored up as reserve food supply in roots, grains and seeds, of which it may amount to 90 per cent. or more of the dry material. In its natural unaltered form it appears as small white granules, which, as we have seen in Chapter II, have definite characteristic forms recognizable microscopically. The granules are insoluble in cold water but

with hot water swell and disintegrate to form a paste. By treatment with acids, the action being greatly accelerated by heating, starch paste loses its colloidal characteristics and is gradually converted into a sirupy mixture of dextrin, maltose and dextrose, finally being entirely changed into the latter.

Dextrin.—The usual method for the formation of dextrin is by the conversion of starch, which may be due to heating, to acids, or to enzyme action. Various forms of dextrin are formed as intermediate products between starch and maltose in the acid conversion of the former, the final product being dextrose. Dextrin, when prepared by diastatic starch conversion, or by heating starch with dilute nitric acid, and purified by precipitating with alcohol, gives a specific rotation of about + 195.

Cellulose.—This is the most prominent single constituent of the vegetable kingdom, although it is rarely found in a pure condition, being present in the walls of cellular tissue combined with lignin and pentosans. Typical percentages of cellulose in the water-free material of various plant substances are tabulated by Browne[1] as follows:

Material (water-free)	Approximate per cent. of cellulose
Wood	60
Bark	40
Straw	40
Leaves	20
Seeds (including husks)	15
Roots, tubers, etc.	10

Pentosans.—These are the parent substances or anhydrides of the pentose sugars, as arabinose and xylose, and are among the chief constituents of vegetable gums and tissues, araban in gum arabic, bran, corn stalks, etc., and xylan in wood gum. The latter is, next to cellulose, the most abundant of plant constituents, comprising from 15 to 30 per cent. of the dry matter of straw, grass, and wood fibers. Boiling dilute acids readily dissolve and hydrolyze the pentosans into the corresponding pentoses, hence the necessity of removing them before determining starch by acid hydrolysis in an ordinary complex vegetable food.

[1] Handbook of Sugar Analysis, p. 575.

Qualitative Tests for Sugars.—It is often desirable before determining quantitatively the amount of sugar or other carbohydrates present in a food material, to learn what particular carbohydrates are present. It should be borne in mind, however, in applying any qualitative tests, that the sugar or other carbohydrate should in general be separated so far as possible in a state of comparative purity, either through its solubility or by other means, before applying the test. Some qualitative tests, as the well-known iodine reaction for starch, can be applied successfully in the presence of large amounts of other substances; with other tests, as the reducing action on copper solutions, quite erroneous conclusions may be drawn from the test unless interfering substances have been shown to be absent.

These qualitative tests may be *general*, applying to a group of sugars, or *specific* and characteristic of a particular sugar. A great many have been described, but the majority of them are applicable only to the pure substance and hence of limited use in the examination of foods.

General Qualitative Tests

The **Molisch**[1] or **α-naphthol reaction** is a widely used general test for carbohydrates. It is described by Mulliken[2] as follows:

Place about 5 mg. of the substance with 10 drops of water in a small, narrow test-tube, and mix with 2 drops of a 10 per cent. chloroform solution of α-naphthol. Allow 1 cc. of pure concentrated sulphuric acid to flow slowly down the side of the tube so as to form a layer beneath the aqueous solution. If a carbohydrate is present, a red ring will appear within a few seconds at the junction of the two layers. The color soon changes on standing or shaking, a very dark purple solution being formed. Shake and allow to stand for 1 or 2 minutes, then add 5 cc. of cold water. In the presence of a carbohydrate, a dull violet precipitate will be formed. Addition of an excess of strong ammonia will change the color to a rusty brown.

Note.—The reaction is assumed to be due to the formation of a

[1] *Monatsh. Chem.*, **1888**, 198.

[2] Identification of Pure Organic Compounds, Vol. I, p. 26.

condensation product between the α-naphthol and the furfural resulting from the decomposition of the carbohydrate.

Fehling's Copper Test for Reducing Sugars.—The reducing sugars may be detected by their action upon alkaline solutions of metallic salts. The monosaccharides, as well as lactose and maltose of the disaccharides that we have discussed, reduce alkaline solutions of metallic salts, as copper or mercury, oxygen being withdrawn and the metal precipitated either as such or as a lower oxide. The metallic salt most commonly employed is one of copper tartrate, known as Fehling's solution, and the method is described as a quantitative one on page 236. It should be noted in using this test as a qualitative one that many substances interfere with the test, either by preventing the precipitation of the cuprous oxide or by precipitating similar substances which might be mistaken for it. It is generally better to follow the precautions prescribed for the quantitative application and remove interfering substances with lead acetate; the excess of lead acetate is then precipitated with potassium oxalate and the clear filtrate tested by adding a few cc. of the solution to 10 cc. of Fehling's solution in a test-tube and boiling 2 minutes. A brick-red precipitate of cuprous oxide will form if reducing sugars are present.

Barfoed's Test.—If the presence of a reducing sugar is shown by the previous test, and it is desired to distinguish lactose or maltose from a monosaccharide, a solution of cupric acetate as suggested by Barfoed[1] may be employed. This is reduced by the latter but not by the former. The reagent is prepared by dissolving 45 grams of neutral crystallized cupric acetate in 900 cc. of water and filtering. To the filtrate is added 1.2 cc. of 50 per cent. acetic acid and the solution diluted to a liter. A portion of the reagent, heated on the water-bath, should show no reduction.

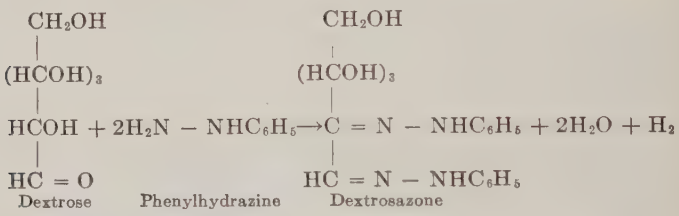
To make the test: To 5 cc. of the reagent, in a test-tube, add 5 cc. of the solution to be tested and place in a boiling water-bath for 3½ minutes; examine for precipitated cuprous oxide, viewing the tube against a black background in a good light. If none is found, let the tube stand at room temperature for

[1] *Z. anal. Chem.*, **1873**, 27; Hinkel and Sherman: *J. Am. Chem. Soc.*, **1907**, 1744.

5 to 10 minutes, pour out the liquid carefully and note if any traces of cuprous oxide remain adhering to the test-tube.

Note.—Hinkel and Sherman found that the test would detect 0.4 milligram of dextrose in the presence of 20 milligrams of lactose or maltose.

Osazone Reaction.—The qualitative test of most general application is the conversion of the sugar into its osazone. Those sugars that have the carbonyl group, that is, the reducing sugars, react with an excess of phenylhydrazine to form osazones. The reaction in the case of dextrose is a typical one:



To carry out the reaction, mix together 1 part of the sugar, 2 parts of phenylhydrazine hydrochloride, 3 parts of crystallized sodium acetate and 20 parts of water in a test-tube. Filter the solution if not perfectly clear, heat in a boiling water-bath for an hour and a half and allow it to cool.

A solution of phenylhydrazine acetate, prepared by adding glacial acetic acid to phenylhydrazine until it is just dissolved, may be used instead of the hydrochloride, if more convenient. In this case the sodium acetate is not necessary.

The osazone thus precipitated is of a yellowish color and more or less crystalline. The osazones of the monosaccharides separate from the hot solution; those of the disaccharides maltose and lactose, only after cooling. It is possible by washing with hot water to separate the two classes nearly completely. The osazone may be purified by filtering on a small paper, washing once with cold water, dissolving in the least possible amount of boiling 50 per cent. alcohol and filtering hot.

In order to identify the osazone thus obtained, its melting point should be determined by the capillary-tube method, as ordinarily used for solid organic compounds. (See page 154.) The principal limitation of the method is shown in the fact that the melting

points of the osazones do not lie very far apart. Dextrose and levulose yield the same osazone, dextrosazone, melting at 204°–205°; galactosazone melts at 191°–196°; lactosazone at 200° and maltosazone at 202°–208° It is possible to distinguish whether the osazone obtained is derived from a pentose, hexose or disaccharide by determining its nitrogen content, or by noting the time required for osazone formation under definite conditions.[1] The osazones of the monosaccharides separate much more quickly than do those of the disaccharides. Under the conditions prescribed by Mulliken, the osazones of the common monosaccharides precipitate in from 2 to 10 minutes, sucrose (due to slight inversion) in half an hour, maltose and lactose not at all from the hot solution. These figures were obtained, however, with the pure sugars. With mixtures, or in the presence of impurities, these times are greatly modified so that the method is of less value.

Special Group Tests

The Furfural Reaction.—The reaction of the pentoses or pentosans with hydrochloric acid, by which furfural is produced, finds considerable application in food analysis, a typical case of its qualitative value being in the detection of artificial invert sugar in honey, page 296. By carefully regulating the conditions, the yield of furfural may be made so nearly constant as to be utilized for the determination of the amount of pentosans, page 264. The reaction takes place through the splitting off of water:

$$\underset{\text{Pentose}}{C_5H_{10}O_5} \rightarrow \underset{\text{Furfural}}{C_5H_4O_2} + \underset{\text{Water}}{3H_2O}$$

To carry out the qualitative test, boil about 5 grams of the material in an Erlenmeyer flask with hydrochloric acid (sp. gr. 1.06). Lay over the mouth of the flask filter paper soaked with anilin-acetate solution.[2] In the presence of furfural, the paper

[1] Maquenne: *Compt. rend.*, **1891,** 799; Mulliken: Identification of Pure Organic Compounds, Vol. I, p. 32; Sherman and Williams: *J. Am. Chem. Soc.*, **1906,** 629.

[2] Prepared by mixing in a test-tube equal volumes of anilin and water and adding glacial acetic acid with constant shaking until the mixture becomes clear. Filter paper is moistened with the solution.

will turn to a brilliant red. Or the boiling with hydrochloric acid may be done in a distilling flask and the distillate tested with phloroglucin, which causes a dark-green precipitate of furfural-phloroglucid.

$$\underset{\text{Furfural}}{C_5H_4O_2} + \underset{\text{Phloroglucin}}{C_6H_6O_3} \rightarrow \underset{\text{Furfural-phloroglucid}}{C_{11}H_8O_4} + H_2O$$

This is the reaction commonly used for the quantitative estimation of more than traces of furfural.

Note.—It should be remembered that many of the hexose carbohydrates, as sucrose or starch, as well as a few other substances, will yield furfural in this reaction, but the amounts are so small as not to interfere seriously with the test for pentoses.

The Resorcin Reaction.[1]—This test, which has already been mentioned on page 123, is of value in distinguishing those sugars having a ketone group, more especially levulose, from the aldehydic sugars as dextrose or lactose.

To make the test, add to 10 cc. of the sugar solution in a test-tube an equal volume of 25 per cent. hydrochloric acid and about 0.1 gram of resorcin and heat gently over a small flame. If a ketose sugar is present, a fiery-red color will be developed.

Notes.—It is important that the test should be carried out as described, especially with respect to the amount of acid. If too much be used, other sugars will give pinkish solutions which might be mistaken for the more pronounced color of the ketoses.

The reason that the reaction is given on page 123 as a test for sucrose in milk is, of course, due to the fact that sucrose, when heated with acid, yields as one of its products levulose, a ketose sugar.

Quantitative Methods

The Determination of Reducing Sugars.—The fact that the reducing sugars, similarly to aldehydes or ketones in general, possess the property of reducing certain alkaline metallic salts has already been noted under the qualitative use of Fehling's solution, page 233.

The reduction of the copper solution may be made quanti-

[1] Seliwanoff: *Ber.*, 1887, 181.

tative for estimating the per cent. of sugar by either (1) determining the volume of sugar solution required to precipitate a measured amount of the copper solution, or (2) by weighing or otherwise determining the cuprous oxide reduced from an excess of the copper reagent by a measured quantity of the sugar solution.

The original Fehling method was of the first type, and these volumetric methods are still largely used in rapid routine work and in such determinations as sugar in urine, but for the general purposes of food analysis are not so well suited as the more accurate gravimetric methods. If it is desired to use the volumetric method, detailed directions for making the test will be found in Sherman's Organic Analysis, 2d Ed., page 70.

Gravimetric Methods.—A great number of gravimetric processes depending upon the use of Fehling's solution have been proposed, nearly all of the older ones being limited to the determination of a single sugar. Of these the best known is Allihn's method for dextrose[1] which is the official method of the Association of Official Agricultural Chemists. By the use of suitable factors, Allihn's table can be used for the determination of other reducing sugars than dextrose,[2] but for general use in food analysis one of the later unified methods for the common reducing sugars is preferable. Of these, the method of Munson and Walker[3] provides a simple procedure with convenient tables for the sugars likely to be met in food products and is the only one which will be described. It has the possible added advantage over the Allihn method that the proportion of alkali used is less and there is correspondingly less tendency toward decomposition of the sugar. A compilation of the more important of the other methods, with their appropriate tables, may be found in Browne's Handbook of Sugar Analysis.

Munson and Walker Method.—*Preparation of Reagent.*—(*a*) *Copper Sulphate.*—Dissolve 34.639 grams of clear crystals of pure copper sulphate ($CuSO_4.5H_2O$), free from efflorescence, in water, add 0.5 cc. of strong sulphuric acid and make up to 500 cc.

(*b*) *Alkaline Tartrate.*—Dissolve 172 grams of the purest

[1] *J. prakt. Chem.* **1880,** 46; *Bur. of Chem., Bull.* **107** (rev.) p. 49.

[2] Browne: *J. Am. Chem. Soc.,* **1906,** 439.

[3] *J. Am. Chem. Soc.,* **1906,** 663; **1907,** 541; **1912,** 202.

Rochelle salts (sodium potassium tartrate) and 50 grams of sodium hydroxide, free from carbonate, in water and make up to 500 cc.

Preparation of Asbestos.—First digest the asbestos, which should be of the amphibole variety, with hydrochloric acid (1:3) for two or three days. Wash free from acid and digest for the same length of time with sodium hydroxide solution (100 grams per liter), after which treat for several hours with hot alkaline copper-tartrate (Fehling's solution). Then wash the asbestos free from alkali, digest it finally with nitric acid (sp. gr. 1.07) for several hours, wash free from acid and keep it in water for use.

Use the asbestos in porcelain Gooch crucibles, having a layer about one-fourth of an inch thick and wash it thoroughly to remove fine particles of asbestos, avoiding the use of too strong suction. Fill the crucible once with alcohol, once with ether, dry for 30 minutes at 100°C., cool in a desiccator and weigh. Do not remove the asbestos after a determination but dissolve the cuprous oxide in hot dilute nitric acid and use the same felts over again, as they improve with use.

The Determination.—Transfer 25 cc. each of the copper and alkaline tartrate solutions to a 400-cc. Jena or Non-sol beaker, and add 50 cc. of the reducing sugar solution, or, if a smaller volume of the sugar solution be used, add enough water to make the final volume 100 cc.

Heat the beaker upon an asbestos gauze over a Bunsen burner, so regulating the flame that boiling begins in 4 minutes, and continue the boiling for *exactly* 2 minutes, keeping the height of the flame the same as in the preliminary boiling. Keep the beaker covered with a watch-glass during the entire time of heating. Without diluting, filter the cuprous oxide at once on an asbestos felt prepared as described above, using suction and a porcelain Gooch crucible. Wash the cuprous oxide thoroughly with water at a temperature of about 60°C., pour the hot solution out of the filter bottle, add 10 cc. of alcohol, and finally 10 cc. of ether. Dry for 30 minutes in a water-oven at 100°C., cool in a desiccator and weigh as cuprous oxide. Find the milligrams of reducing sugar corresponding to the weight of

cuprous oxide from the appropriate column in Table XXXIV, page 241.

Notes.—The reagents should be the purest obtainable and the solutions, if turbid, should be filtered through glass wool until perfectly clear. The solution and the asbestos should be tested by making "blank" determinations according to the regular procedure, except that no sugar is present. The crucible should not lose or gain in weight in a blank determination by more than 0.2 milligram.

Close adherence to the details of the procedure as regards the volume of solution and time of heating is essential. The reduction of the copper solution is not complete, more copper being reduced if the solution is more concentrated or the time of heating prolonged. Hence the given volume must be used, the solution boiled for exactly 2 minutes, making 6 minutes for the total time of heating, and the cuprous oxide filtered as soon as possible. In carrying out the method for the first time, it is advisable to regulate the flame for the 4-minute heating by a preliminary trial with 100 cc. of water.

The volume of sugar solution used in a determination should not contain, in general, more than 0.220 gram of a monosaccharide, 0.300 gram of lactose or 0.350 gram of maltose.

In many food products, the reducing sugar determination is complicated by the presence of sucrose. Although sucrose does not reduce Fehling's solution directly, it is hydrolyzed to some extent by the hot Fehling's solution and thus gives small amounts of reducing sugar. The extent of this reducing action of sucrose is dependent upon the concentration of the sucrose and upon the amount of unreduced copper. Consequently, in accurate determinations of reducing sugar, it may be necessary to take the reducing action of the sucrose into consideration. The error may be avoided by taking an amount of the sugar solution such that the invert sugar will reduce nearly all of the copper, under which conditions the inversion of the sucrose is so slight as to be negligible. Another method, and the one adopted by Munson and Walker, is to determine and tabulate the copper reduced by invert sugar in the presence of varying amounts of sucrose, separate columns being given in Table XXXIV, page 241, for 0.4 gram and for 2 grams total weight of sucrose and invert sugar. It is

generally possible from the other determinations made, as solids, ash and polarization, to calculate how much of the material should be employed in the determination of reducing sugar in order that the total sugar shall be approximately one of the values noted. In other columns of the table are given the copper values for mixtures of sucrose and lactose, the proportions chosen being such as to be of value in the analysis of condensed milk and of milk chocolate, respectively.

The use of a table for calculating the amount of reducing sugar corresponding to a given amount of cuprous oxide is necessary on account of the variation in reducing power of the sugars with the concentration. In general, the reducing power of the monosaccharides decreases with increased concentration and the same is true to a less extent of the disaccharides. The latter, however, are more subject to variations due to slight changes in the conditions attending the reduction, so that a definite law cannot be so well established for them.

The method of weighing the reduced copper as cuprous oxide, while convenient and accurate with sugars of reasonable purity, is not exact when working with impure solutions such as molasses and sirups. With these, organic or mineral impurities may be carried down with the precipitated copper, or a part of the copper may be precipitated in organic combinations rather than as the oxide. This condition can usually be determined by inspection of the cuprous oxide precipitate in the crucible. If it is not of the characteristic bright red color, but appears dull brown or various shades of yellow or greenish red, the result would better be checked by igniting to cupric oxide or by dissolving the precipitate and determining its content of copper.

Results of considerably greater accuracy may be obtained by igniting the precipitate of cuprous oxide and weighing it as cupric oxide. This may be done by heating the crucible in a muffle at a red heat for 15 minutes or more simply by placing the crucible in a slightly larger one of platinum or nickel and heating the outer crucible to bright redness for the same length of time. The crucible is then transferred to a desiccator and weighed when cool, bearing in mind the somewhat hygroscopic nature of cupric oxide. To convert cupric oxide into cuprous

TABLE XXXIV.—MUNSON AND WALKER'S TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL SUGAR), LACTOSE, LACTOSE AND SUCROSE (2 MIXTURES) AND MALTOSE (CRYSTALLIZED)

Cuprous oxide (Cu_2O), mgm.	Copper (Cu), mgm.	Dextrose, mgm.	Invert sugar, mgm.	Invert sugar and sucrose		Lactose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Lactose and sucrose		Maltose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Cuprous oxide (Cu_2O), mgm.
				0.4 gm. total sugar, mgm.	2 gm. total sugar, mgm.		1 lactose, 4 sucrose, mgm.	1 lactose, 12 sucrose, mgm.		
10	8.9	4.0	4.5	1.6	.....	6.3	6.1	.....	6.2	10
15	13.3	6.2	6.7	3.9	.....	9.4	9.1	.....	10.4	15
20	13.8	8.3	8.9	6.1	.....	12.5	12.1	.....	14.6	20
25	22.2	10.5	11.2	8.4	.....	15.7	15.2	.....	18.7	25
30	26.6	12.6	13.4	10.7	4.3	18.8	18.2	.....	22.9	30
35	31.1	14.8	15.6	12.9	6.5	22.1	21.3	.....	27.1	35
40	35.5	16.9	17.8	13.2	3.8	25.5	24.7	.....	31.3	40
45	40.0	19.1	20.1	17.5	11.1	28.9	28.0	.....	35.4	45
50	44.4	21.3	22.3	19.7	13.4	32.3	31.3	.....	39.6	50
55	48.9	23.5	24.6	22.0	15.7	35.8	34.6	.....	43.8	55
60	53.3	25.6	26.8	24.3	18.0	39.2	37.9	.....	48.0	60
65	57.7	27.8	29.1	26.6	20.3	42.6	41.3	.....	52.1	65
70	62.2	30.0	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
75	66.6	32.2	33.6	31.2	24.9	49.4	47.9	44.8	60.5	75
80	71.1	34.4	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
85	75.5	36.7	38.2	35.8	29.6	56.3	54.6	50.7	68.8	85
90	79.9	38.9	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
95	84.4	41.1	42.7	40.5	34.2	63.2	61.3	56.6	77.2	95
100	88.8	43.3	45.0	42.8	36.6	66.6	64.6	59.6	86.3	100
105	93.3	45.5	47.3	45.2	38.9	70.0	68.0	62.6	85.5	105
110	97.7	47.8	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
115	102.2	50.0	51.9	49.8	43.6	76.9	74.6	68.5	93.9	115
120	106.6	52.3	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
125	111.0	54.5	56.6	54.5	48.3	83.8	81.3	74.5	102.2	125
130	115.5	56.8	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
135	119.9	59.0	61.2	59.3	53.1	90.6	88.1	80.5	110.5	135
140	124.4	61.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
145	128.8	63.6	65.9	64.0	57.8	97.5	94.8	86.5	118.9	145
150	133.2	65.9	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
155	137.7	68.2	70.6	68.8	62.6	104.4	101.5	92.6	127.2	155

TABLE XXXIV.—MUNSON AND WALKER'S TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL SUGAR), LACTOSE, LACTOSE AND SUCROSE (2 MIXTURES) AND MALTOSE (CRYSTALLIZED).—(*Continued*)

Cuprous oxide (Cu_2O), mgm.	Copper (Cu), mgm.	Dextrose, mgm.	Invert sugar, mgm.	Invert sugar and sucrose		Lactose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Lactose and sucrose		Maltose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Cuprous oxide (Cu_2O), mgm.
				0.4 gm. total sugar, mgm.	2 gm. total sugar, mgm.		1 lactose, 4 sucrose, mgm.	1 lactose, 12 sucrose, mgm.		
160	142.1	70.4	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
165	146.6	72.8	75.3	73.6	67.4	111.3	108.2	98.6	135.5	165
170	151.0	75.1	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
175	155.5	77.4	80.1	78.4	72.2	118.2	114.9	104.7	143.9	175
180	159.9	79.7	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
185	164.3	82.0	84.9	83.2	77.1	125.1	121.7	110.7	152.2	185
190	168.8	84.3	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
195	173.2	86.7	89.6	88.0	81.9	132.0	128.5	116.8	160.5	195
200	177.7	89.0	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
205	182.1	91.4	94.5	92.9	86.8	138.9	135.3	122.9	168.9	205
210	186.5	93.7	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
215	191.0	96.1	99.3	97.8	91.7	145.8	142.0	129.0	177.2	215
220	195.4	98.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
225	199.9	100.8	104.2	102.7	96.6	152.7	148.8	135.2	185.5	225
230	204.3	103.2	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
235	208.7	105.6	109.1	107.7	101.6	159.6	155.6	141.3	193.8	235
240	213.2	108.0	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
245	217.6	110.4	114.0	112.6	106.5	166.6	162.4	147.5	202.2	245
250	222.1	112.8	116.4	115.1	109.1	170.1	165.8	150.6	206.3	250
255	226.5	115.2	118.9	117.6	111.5	173.5	169.2	153.7	210.5	255
260	231.0	117.6	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
265	235.4	120.0	123.9	122.6	116.5	180.5	176.0	159.9	218.8	265
270	239.8	122.5	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
275	244.3	124.9	128.9	127.7	121.6	187.4	182.9	166.1	227.1	275
280	248.7	127.3	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
285	253.2	129.8	133.9	132.7	126.6	194.4	189.7	172.4	235.5	285
290	257.6	132.3	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
295	262.0	134.7	138.9	137.8	131.7	201.3	196.5	178.7	243.8	295
300	266.5	137.2	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
305	270.9	139.7	144.0	142.9	136.8	208.3	203.3	185.0	252.1	305
310	275.4	142.2	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
315	279.8	144.7	149.1	148.1	141.9	215.3	210.2	191.3	260.4	315
320	284.2	147.2	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
325	288.7	149.7	154.3	153.2	147.1	222.2	217.0	197.6	268.7	325

TABLE XXXIV.—MUNSON AND WALKER'S TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL SUGAR), LACTOSE, LACTOSE AND SUCROSE (2 MIXTURES) AND MALTOSE (CRYSTALLIZED).—(*Continued*)

Cuprous oxide (Cu_2O), mgm.	Copper (Cu), mgm.	Dextrose, mgm.	Invert sugar, mgm.	Invert sugar and sucrose		Lactose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Lactose and sucrose		Maltose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Cuprous oxide (Cu_2O), mgm.
				0.4 gm. total sugar, mgm.	2 gm. total sugar, mgm.		1 lactose, 4 sucrose, mgm.	1 lactose, 12 sucrose, mgm.		
330	293.1	152.2	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330
335	297.6	154.7	159.4	158.4	152.3	229.2	223.9	204.0	277.0	335
340	302.0	157.3	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340
345	306.5	159.8	164.6	163.7	157.5	236.2	230.8	210.3	285.4	345
350	310.9	162.4	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350
355	315.3	164.9	169.8	168.9	162.7	243.2	237.7	216.7	293.7	355
360	319.8	167.5	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360
365	324.2	170.1	175.1	174.2	167.9	250.2	244.6	223.1	302.0	365
370	328.7	172.7	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370
375	333.1	175.3	180.4	179.5	173.2	257.2	251.5	229.6	310.3	375
380	337.5	177.9	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380
385	342.0	180.5	185.7	184.8	178.5	264.2	258.5	236.0	318.6	385
390	346.4	183.1	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390
395	350.9	185.7	191.0	190.2	183.9	271.2	265.4	242.5	326.9	395
400	355.3	188.4	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400
405	359.7	191.0	196.4	195.6	189.2	278.2	272.3	249.0	335.2	405
410	364.2	193.7	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
415	368.6	196.3	201.8	201.0	194.6	285.3	279.3	255.5	343.5	415
420	373.1	199.0	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
425	377.5	201.7	207.3	206.5	200.0	292.3	286.3	262.1	351.8	425
430	382.0	204.4	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
435	386.4	207.1	212.8	212.0	205.5	299.3	293.3	268.7	360.1	435
440	390.8	209.8	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
445	395.3	212.5	218.3	217.5	211.0	306.3	300.3	275.3	368.4	445
450	399.7	215.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
455	404.2	218.0	223.9	223.0	216.5	313.4	307.3	281.9	376.7	455
460	408.6	220.7	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
465	413.0	223.5	229.5	228.6	222.0	320.4	314.3	288.5	385.0	465
470	417.5	226.2	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
475	421.9	229.0	235.1	234.2	227.6	327.5	321.2	295.2	393.3	475
480	426.4	231.8	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
485	430.8	234.6	240.8	239.9	233.2	334.5	328.2	301.8	401.6	485
490	435.3	237.4	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490

oxide, multiply by the factor 0.8994. It is of course necessary that the crucible and asbestos should have been previously ignited and weighed under similar conditions.

A still more accurate method is to dissolve the precipitate of cuprous oxide and determine the copper volumetrically or by electrolysis. The volumetric method most commonly employed is that depending upon the liberation of iodine from potassium iodide, the reaction for cupric acetate being:

$$2Cu(C_2H_3O_2)_2 + 4KI \rightarrow Cu_2I_2 + 4KC_2H_3O_2 + I_2$$

The procedure proposed by Low[1] has been adopted by the Association of Official Agricultural Chemists as a provisional method[2] but the modification suggested by Peters[3] is more rapid and convenient.

Peters' Modified Volumetric Iodide Method.—*Procedure.*—Dissolve the cuprous oxide in the crucible in 5–10 cc. of concentrated nitric acid to which one-half its volume of water has been added. This is best done by covering the crucible with a small watch-glass and allowing the acid to run beneath it from a pipette. After solution wash the crucible with 25 cc. of water, added in small portions. Rinse the solution into a 250-cc. Erlenmeyer flask, keeping the volume as small as possible, add 1 gram of pure powdered talc and boil vigorously for 5 to 10 minutes. Cool to room temperature, make slightly ammoniacal and then slightly acid with acetic acid. Add 10 cc. of a saturated potassium iodide solution and titrate with $\frac{N}{10}$ sodium thiosulphate until the brownish tinge has become faint, then add a few cubic centimeters of starch solution and continue the titration until the blue color due to iodine disappears. Toward the end of the titration, the blue color changes to a faint lilac and the addition of the thiosulphate should be made rather slowly, drop by drop until the solution clears. One or two trials will ensure a satisfactory end-point.

Notes.—The chief objection to the iodide method, as formerly carried out, was in the danger of incomplete removal of the

[1] *J. Am. Chem. Soc.*, **1902**, 1082.
[2] *Bur. of Chem., Bull.* **107** (revised), p. 241.
[3] *J. Am. Chem. Soc.*, **1912**, 422.

nitrous acid formed in the solution of the cuprous oxide. The very vigorous boiling and many minute bubbles caused by the addition of the finely powdered talc serve to remove the nitrous acid completely.

The thiosulphate solution employed should be standardized against a solution of pure copper in exactly the same manner as in a regular determination.

Electrolytic Method.—For occasional determinations of the reduced copper, or in cases where the longer time required is not objectionable, the electrolytic method is convenient and exact.

Transfer the asbestos and the cuprous oxide by means of a glass rod to a beaker and rinse the crucible with about 30 cc. of a boiling mixture of dilute sulphuric and nitric acids, containing 65 cc. of sulphuric acid (sp. gr. 1.84) and 50 cc. of nitric acid (sp. gr. 1.42) per liter. Heat and shake until solution is complete, filter and wash. Return the asbestos to the crucible to be used for subsequent determinations. Electrolyze the solution, conveniently over night, using a current density of about 0.25 ampere, or as described under the electrolytic determination of copper in any standard text-book on analytical chemistry.

Determination of Sucrose by Fehling's Solution.—Although in the majority of cases sucrose is most quickly and conveniently determined by the polariscope, in the absence of such an instrument, it may be determined with equal accuracy by means of Fehling's solution. As a matter of fact, in some foods containing only a small percentage of sucrose, and a comparatively large amount of reducing sugars, as in honey, the determination by means of copper reduction is more exact than the optical method.

Since sucrose has no reducing power, it is, of course, necessary first to convert it to reducing sugar. This may be done by hydrolyzing it with dilute hydrochloric acid, 95 parts of sucrose yielding 100 parts of invert sugar. If then the resulting invert sugar be determined by Fehling's solution, 95 per cent. of the weight of invert sugar will be the corresponding weight of sucrose. If reducing sugars are present as well as sucrose, as is commonly the case in foods, the sucrose must be determined from the difference in reducing power before and after

inversion. The method employed and the calculation can be best illustrated by an example.

Suppose that it is desired to determine sucrose in a raspberry sirup containing sucrose and invert sugar. Five grams of sirup are dissolved and made up to 500 cc. (Solution *A*). Another 5 gram portion is weighed, dissolved in about 75 cc. of water and inverted according to the Herzfeld method (page 257). The inverted solution, after cooling, is nearly neutralized with sodium hydroxide, care being taken to leave it faintly acid, and made up also to 500 cc. (Solution *B*). In an aliquot part of each solution the reducing sugar is determined by the Munson and Walker method, page 237, calculating the results in each case as invert sugar.

Example.—

		Mgm. copper		Mgm. invert sugar
50 cc. of Solution B	=	407.7	=	226.1
50 cc. of Solution A	=	195.4	=	101.7
		212.3		124.4

$124.4 \times 0.95 = 118.2$ mgm. = 23.64 per cent. sucrose
101.7 mgm. = 20.34 per cent. invert sugar

If in any particular case the reducing sugar present should be some other sugar than invert sugar, it is still necessary in this determination to calculate it as invert sugar in order to subtract the amount from the total to find the inverted sucrose.

Care should be taken to employ such an aliquot part for the final reduction that the amount of invert sugar present shall not exceed 240 mgm., which is practically the limit of the Munson and Walker table. It may be necessary to determine the correct amount to use by a preliminary determination.

Optical Methods

An important class of methods for determining the carbohydrates is based on their behavior toward polarized light. All of the natural carbohydrates, as is true in general of organic substances possessing one or more asymmetric carbon atoms, possess the power of rotating the plane of polarized light and under definite conditions this may be used as a measure of their quantity.

Specific Rotation.—The unit in measurements of optical rotation is the specific rotating power of the substance or its *specific rotation*. This may be defined as the rotation in angular degrees of the plane of polarized monochromatic light which is produced by a solution of the optically active substance having a concentration of 1 gram in 1 cc. and 1 dm. long. This is given by the expression

$$[\alpha] = \frac{av}{lw}$$

where $[\alpha]$ is the specific rotation, a the degrees of angular rotation, l the length of the column of solution in decimeters, and w the grams of substance in v cc. of solution. If the concentration is expressed in percentage of substance in solution, as is sometimes the case, the formula is stated differently. Calling the percentage (grams of substance in 100 grams of solution) p and the specific gravity d, $w = pd$, and $v = 100$, hence

$$[\alpha] = \frac{100a}{lpd}.$$

The amount of angular rotation under the conditions stated depends further on the wave length of the light employed, the concentration and the temperature, and certain of these variables are customarily stated in expressing the value for any given substance. Thus the expression $[\alpha]_D^{20}$ means the specific rotation as determined for the *D*-ray of the spectrum and at 20°C., these being the standard conditions for measurement.

The following values for $[\alpha]_D^{20}$ of the carbohydrates commonly occurring in foods will be found sufficiently exact for laboratory calculations:

Arabinose	+104.5
Dextrose	+ 52.5
Levulose	− 92.5
Invert sugar	− 20.0
Lactose	+ 52.5
Galactose	+ 80.5
Maltose	+138.5
Sucrose	+ 66.5
Xylose	+ 19.0
Starch, Dextrin	about + 195 for purified material.

Effect of Temperature and Concentration upon Specific Rotation.—Variations in the concentration of the solution and in the temperature at which the observation is made have with many sugars a pronounced effect on the specific rotation. In the presence of invert sugar, for example, accurate polariscopic determinations of the sugar are impossible unless made at known temperatures. In general, the sugars show a decrease in specific rotation with increased temperature, the change being especially marked with levulose and arabinose. Xylose, on the other hand, shows a slight increase and dextrose remains apparently unchanged at temperatures between 0° and 100°C.

The table below, arranged from Browne's Handbook of Sugar Analysis, gives the correction for concentration or for concentration and temperature of the more common sugars:

Sucrose[1]	$[\alpha]_D^{20} = +66.435 + 0.00870c - 0.000235c^2$ (c = 0 to 65 grams per 100 cc.)
Dextrose[2]	$[\alpha]_D^{20} = +52.50 + 0.018796p + 0.00051683p^2$ (p = 0 to 100 per cent.)
Levulose[3]	$[\alpha]_D^t = -[101.38 - 0.56t + 0.108\,(c - 10)]$
Invert sugar	$[\alpha]_D^t = -[27.9 - 0.32t]$
Lactose[4]	$[\alpha]_D^t = +52.53 - 0.07\,(t - 20)$ [t = 15° to 25°C.]
	(+ 52.53 = constant for c = 2.4 to 40)
Maltose[5]	$[\alpha]_D^t = 140.375 - 0.01837p - 0.095t$.

As stated above, there is no temperature correction for dextrose, and the temperature and concentration corrections for sucrose are so slight as to be negligible in food analysis.

Mutarotation.—The reducing sugars when freshly dissolved often give at first different polarization values which gradually change and become constant after some hours. For example, dextrose when first dissolved may give a rotation value of 105.2 instead of the constant value 52.5. This phenomenon is explained by the existence of two differing arrangements of the atoms within the sugar molecule, one configuration giving a high-rotating and

[1] Landolt: "Das optische Drehungsvermögen" (1898), p. 420.
[2] Tollens: *Ber.*, **1884**, 2238.
[3] Jungfleisch and Grimbert: *Compt. rend.*, **1888**, 390.
[4] Schmoger: *Ber.*, **1880**, 1922.
[5] Meissl: *J. prakt. Chem.*, **1892**, 114.

the other a low-rotating form of the sugar. The constant rotation form is thus a mixture of the two in equilibrium.[1]

The constant rotation can be obtained by letting the solution stand for some hours, or instantaneously by bringing to a boil or adding about 0.1 per cent. of ammonia, the change being greatly accelerated by heat or by acids and alkalies. The possibility of mutarotation should be borne in mind when polarizing such food substances as honey or milk.

The Polariscope.—The polariscope consists essentially of a device for producing plane polarized light, ordinarily two specially prepared prisms of calc spar (Nicol prisms), one of which must be capable of rotation and be fixed to a suitable scale by which its angle of rotation can be measured. Between these prisms is placed a tube containing the solution to be ex-

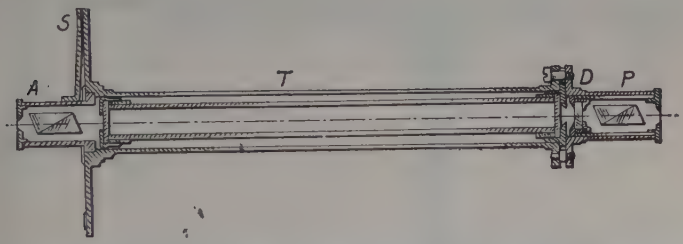


FIG. 45.—Essential parts of polariscope. (ROLFE.) *A*, Revolving sleeve containing eyepiece and analyzer; *S*, scale showing position of rotation of analyzer; *T*, tube of optically active solution; *P*, polarizer; *D*, end-point device.

amined. In order to make the instrument sufficiently exact for practical work, some end-point device is necessary. The arrangement of these essentials is indicated in Fig. 45.

Construction of Polariscopes.—The principal difference in the various polariscopes lies in the form of end-point device employed. Of these the most important are:

(*a*) *The Jellet-Cornu "split" prism*, which consists of an ordinary Nicol prism which has been bisected lengthwise and small wedge-shaped sections removed, after which the prism is cemented together again. The effect of this is to slightly incline toward each other the planes of polarization of the light illuminat-

[1] For a full review and bibliography of the subject of "Mutarotation," see Hudson, *J. Am. Chem. Soc.*, **1910**, 889.

ing the two halves of the field. The split prism being used as a polarizer, the field produced by rotating the analyzer until the Nicols are "crossed" will not be black, as would be the case with ordinary prisms, but shows a faint uniformly lighted field or zero point. The slightest rotation of the analyzer from this position causes a shading in one-half of the field.

(*b*) *Laurent Polariscope.*—In this instrument the arrangement of the prisms is as usual, the only differences being in the provision for a slight rotation of the polarizer to the right or left, and

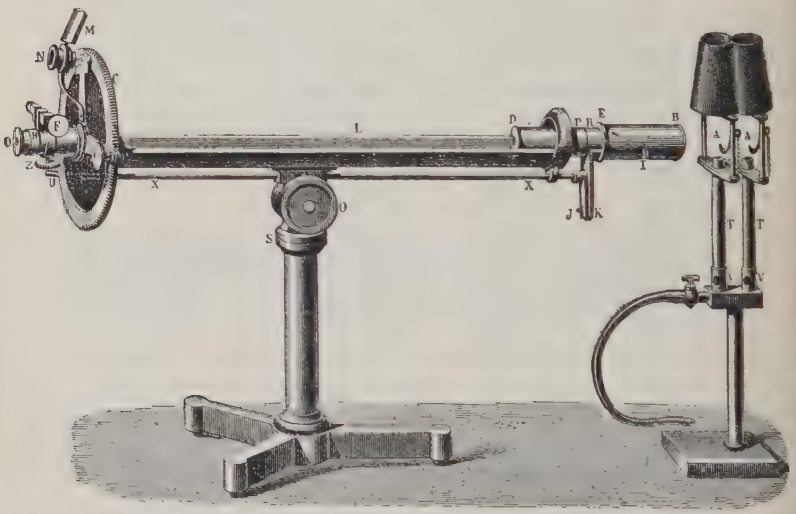


FIG. 46.—LAURENT polariscope.

in the end-point device. This latter consists of a thin plate of quartz cut parallel to its optical axis. Such a section of quartz is doubly refracting and the thickness is such that one component of the light has its vibrations accelerated half a wave length. The effect of this is the same as with the Jellet-Cornu prism, giving two planes of vibration which are inclined toward each other in each half of the field by a small angle, but with the decided advantage that this angle can be varied by means of the rocking polarizer. Such an instrument, which is well suited for general requirements, is shown in Fig. 46. Its principal disadvantage is that it requires monochromatic light.

(c) *The Lippich Polarizer.*—The shadow device proposed by Lippich consists of a small Nicol prism mounted in front of the rotating polarizer so as to cover one-half of the field. The angle can be changed as in the Laurent form. The Lippich polarizer is probably the most sensitive form, but is somewhat more complicated than the Laurent and has the disadvantage that the zero-point of the analyzer must be changed for every change in the sensitive angle of the polarizer.

The Saccharimeter.—More generally used in food laboratories than the rotary polariscopes or polarimeters, just described, are the commercial forms graduated to read directly in percentage of sugar and hence called *saccharimeters*. In these the polarizer and analyzer are stationary and the rotation caused by the sugar solution is measured by interposing a wedge of optically active quartz until the rotation of the sugar is exactly compensated by that of the quartz. Readings are made on a scale attached to the quartz wedge. White light may be used in place of monochromatic since the dispersive power of quartz and of sugar in water solution is very nearly the same.

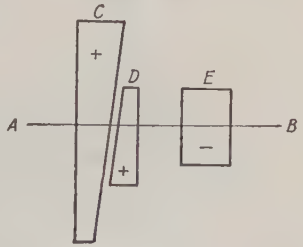


FIG. 47.—Diagram of quartz-wedge compensator. (ROLFE.)

The arrangement of the optical parts in the simpler form of quartz-wedge compensator, a single wedge system, will be clear from the diagram, Fig. 47. *AB* represents the line of vision, the eye of the observer being at *A*. *C* and *D* are two wedges of dextro-rotatory quartz, of which *C* is movable and *D* not. *E* is a fixed section of levo-rotatory quartz. The two wedges together make a section of parallel sides to *E*, the thickness of which can be varied by moving *C*. At the zero point the combined thicknesses of *C* and *D* are equal to *E*. If, however, a tube of dextro-rotatory sugar solution be placed in the instrument between the polarizer and the compensation plate *E*, it will be necessary to restore the optical neutrality by moving *C* until sufficient less dextro-rotatory quartz is interposed to compensate for the dextro-at 17.5°C., of the sugar. If the sugar solution should be levo-ro-meter is the vill of course be necessary to move *C* in the opposite

Mohr cc. = 1.002

direction. The quartz wedges can be equally well *levo-rotatory*, in which case the compensation plate *E* must be dextro-rotatory.

A more complicated form is the double-wedge system, in which the compensation plate is replaced by another pair of wedges of

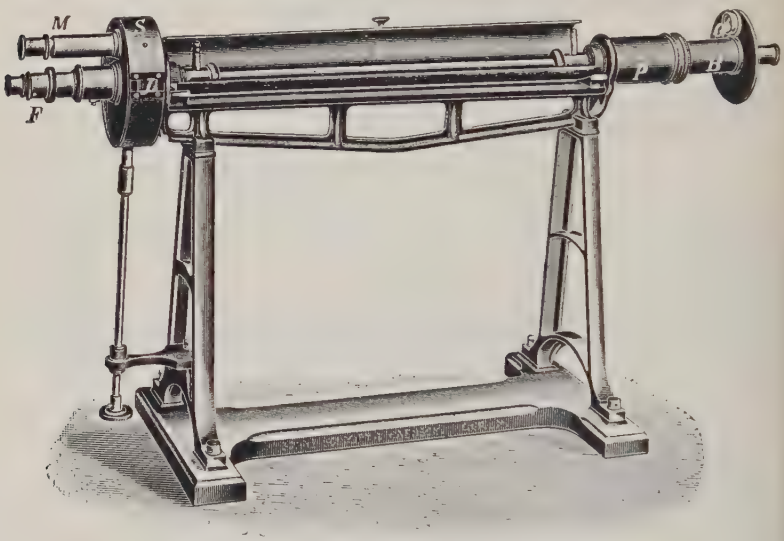


FIG. 48.—Quartz-wedge saccharimeter.

opposite rotation. This has the advantage that any reading obtained by the working wedge can be verified by a second reading taken on the control wedge.

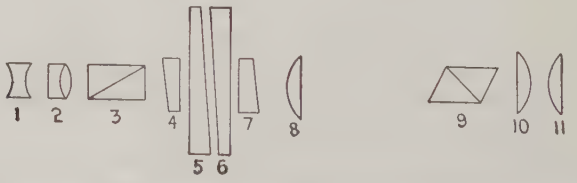


FIG. 49.—Diagram of saccharimeter with double-wedge compensator. (WIECHMANN.)

A general idea of the appearance of the saccharimeter may be gained from Fig. 48, which shows a standard type of instrument in use in many laboratories, and the arrangement of the parts is well shown in Fig. 49, which has reference in this case to the principal double-wedge compensator.

Beginning at the left of the figure, which is the end of the instrument nearest the observer, 1 and 2 are the eyepiece and objective, respectively, of the telescope through which the field is viewed; 3 is the Nicol prism or analyzer; the two pairs of quartz wedges, 4, 5, and 6, 7, constitute the double-wedge compensator; 8 is a lens; 9 is the second Nicol prism or polarizer, the tube of solution being inserted between 8 and 9; 10 and 11, at the end nearest the source of light, are the collimating lenses.

The end-point device in the saccharimeter is the same as in the polariscopes previously mentioned, the Jellet-Cornu prism and the Lippich polarizer being the forms most frequently employed.

Any convenient source of illumination, which furnishes a steady white light, may be used. A high candle power tungsten lamp with coiled filament is perhaps the best, although the incandescent gas lamp (Welsbach) is also excellent. In either case a plate of ground glass should be interposed between the lamp and the instrument in order to diffuse the light and give a uniform field.

For an extended discussion of the construction of polariscopes and saccharimeters, the student is referred to the following special treatises:

Browne.—Handbook of Sugar Analysis.
Landolt.—Optical Rotation of Organic Substances (Translation by Long).
Rolfe.—The Polariscope in the Chemical Laboratory.

Scale and Normal Weight.—The scale of a saccharimeter is so graduated as to read 100° when a certain definite weight (the *normal weight*) of pure sucrose is dissolved in 100 cc. at a definite temperature and polarized in a 200-mm. tube at the same temperature. This standard was fixed originally by Ventzke as the rotation of a solution of sucrose having a specific gravity of 1.100 at 17.5°C., corresponding to 26.048 grams of sucrose in 100 cc.

Since then the saccharimeters in common use have been variously graduated for 26.048 grams of sucrose in 100 *Mohr* cubic centimeters; for 26.048 grams of sucrose in 100 *true* cubic centimeters;[1] and finally, following the recommendations of the

[1] The Mohr cubic centimeter is the volume occupied by 1 gram of water at 17.5°C., weighed in air with brass weights; the true or metric cubic centimeter is the volume of 1 gram of water at 4°C., weighed *in vacuo*. (1 Mohr cc. = 1.00234 true cc.)

International Sugar Commission (1900), for 26.000 grams of sucrose in 100 *true* cubic centimeters, which is practically the same as the first. It is therefore essential that the exact standard of the instrument used should be known, the possible error from neglect of this precaution amounting to over 0.2 per cent.

The value of 1° on the Ventzke scale of the saccharimeter in degrees of angular rotation is 0.3466 for carefully purified light. Under ordinary laboratory conditions, however, the value may differ slightly from this, being dependent upon the temperature and upon the difference in dispersive power of the solution polarized and that of quartz.

Bichromate Light Filter.—When polarizing solutions of some sugars, even with pure sucrose at high rotation, the two halves of the saccharimeter field show slight differences of tint at the zero-point. This is due to the fact that these solutions are not of exactly the same dispersive power as quartz and causes slight differences in readings with different observers. This error can be readily eliminated by passing the light through a cell of potassium bichromate solution, placed in the end of the saccharimeter nearest the light. The proper strength is a 3 per cent. solution of potassium bichromate in a cell 3 cm. long. For polarizing solutions containing commercial glucose[1] a bichromate cell is necessary for accurate work, and it is advantageous to use it in all polarizations of food products. If the polariscope available is not provided with a cell for bichromate solution, an aurantia screen placed between the lamp and the instrument will be found a very fair substitute.[2]

Determination of Sugar by the Polariscope.—*Preparation of the Solution.*—Weigh out the normal weight of the sample, conveniently in a "sugar dish" (Fig. 50). Weigh quickly to avoid loss by evaporation and weigh only to the nearest 0.005 gram. Transfer the weighed sample by means of a small amount of water to a properly calibrated 100-cc. flask. The total volume should not exceed 60–65 cc. Dissolve by shaking, and if the

[1] For such material, Browne (*Bur. of Chem., Bull.* **122**, p. 221) prefers a solution of twice the above concentration.

[2] Such a screen may be easily made by taking a small photographic plate, fixing it in hyposulphite without exposure to light, washing and soaking it in an alcoholic solution of the dye aurantia for 10–15 minutes. The plate is then rinsed and dried.

solution is not perfectly clear and nearly free from color, clarify it by adding 2 to 5 cc. of basic lead acetate or occasionally 6 to 10 cc. of alumina cream.[1] It is sometimes preferable to add both, the alumina cream serving to precipitate the excess of lead. In any case it is desirable to use only as much clarifier as is absolutely necessary since an excess may cause serious error, as explained on page (256). When the right amount of lead has been added, a flocculent precipitate usually settles, leaving a clear solution above. Make up to the 100-cc. mark at 20°C. and mix thoroughly. If foam obscures the meniscus, a drop or two of ether will remove it. Filter the solution through a dry filter, rejecting the first few cubic centimeters of the filtrate, and keeping the funnel covered with a watch-glass to avoid evaporation. Rinse the polariscope tube twice with small portions of the solution and fill it



FIG. 50.—Sugar dish for weighing samples.

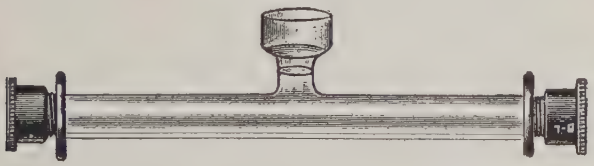


FIG. 51.—Polariscope tube with opening for thermometer.

so that no air-bubbles remain. See that the outside of the cover-glasses is clean and dry and examine the solution by looking through the tube lengthwise before placing it in the instrument. If the solution is not perfectly clear and bright,

[1] These reagents are prepared according to the methods of the Association of Official Agricultural Chemists (*Bur. of Chem., Bull.* **107,** Revised, p. 40) as follows:

1. "Basic Lead Acetate.—Prepare by boiling 430 grams of normal lead acetate, 130 grams of litharge, and 1000 cc. of water for half an hour. Allow the mixture to cool and settle and dilute the supernatant liquid to 1.25 sp. gr. with recently boiled water. Dry lead subacetate may be substituted for the normal salt and litharge, if desired."

2. "Alumina Cream.—Prepare a cold saturated solution of alum in water and divide into two unequal portions. Add a slight excess of ammonium hydroxide to the larger portion and then add by degrees the remaining alum until the mixture is just acid to litmus paper."

it will be useless to try to read it. Since levulose is quite common in food products, it is generally best to make all polarizations in a tube provided with an opening on the side through which a thermometer can be inserted to take the temperature, as shown in Fig. 51.

Zero Error.—It is seldom that the instrument reads exactly zero, so any deviation must first be determined.

See that the instrument is pointed directly at the source of light, which should be about a tube length (200 mm.) from the end of the saccharimeter. Set the scale a few divisions from zero and focus the eyepiece so that the field and the dividing line are perfectly clear and sharp. Rotate the milled head until the field is uniformly illuminated. It is usually best to do this by moving it very slightly on either side of the zero-point and observing when the shadow just flits across the dividing line. Take the average of six separate readings made in this way as the *zero error.*

Polarization of the Solution.—This is made at 20°C. in the manner just described for the zero reading, taking the average of six readings, and correcting for the zero error.

Notes.—In thus measuring a physical property readily affected by changes of concentration and temperature, certain precautions are absolutely necessary. Care should be taken to avoid loss by evaporation during weighing and filtration; changes of temperature by close proximity of heat to the instrument or by careless handling of the observation tube must be carefully prevented.

By far the greater source of error, however, especially with the complex mixtures that may be met in foods, is in clarification. These errors are due mainly to the use of basic lead acetate in excessive amount and are (1) volume occupied by the lead precipitate, increasing the concentration of the solution; (2) precipitation of basic lead salts of dextrose and levulose; (3) change in specific rotation of levulose due to the formation of a soluble dextro-rotatory lead levulosate. The error due to volume of precipitate is ordinarily neglected in food work. If desired, however, it can be estimated and corrected by the method of double dilution[1] as illustrated on page 115, or dry lead subacetate may

[1] Scheibler: *Z. Ver. Deut. Zuckerind,* **1875,** 1054.
See Browne: Handbook of Sugar Analysis, p. 209.

be substituted for the solution generally used. (Horne's dry defecation method.[1]) The other two sources of error are obviously of less consequence if the sucrose alone is to be determined by double polarization (see below) as is customary in food analysis. In any case, however, only the smallest amount of basic lead acetate necessary to clarify the solution should ever be used. The quantities generally employed do not affect the specific rotation of sucrose.

Clerget's or Double Polarization Method.—In the presence of other optically active substances, the percentage of sucrose evidently cannot be determined by the simple polarization method just described. In nearly all saccharine food products other sugars than sucrose, some dextro, others levo-rotatory, are present, so that in general the more complicated double polarization methods must be used. These depend upon measuring the *change* in rotation produced by hydrolysis of the sugar solution, the conditions being such that sucrose alone shall be hydrolyzed.

By hydrolysis the sucrose is changed to invert sugar. A solution of the normal weight of sucrose in 100 cc., polarizing + 100° on the Ventzke scale, will, when inverted by the method described below, polarize − 42.66° at 0°C.

Since the levo-rotation of invert sugar decreases practically 0.5° for each degree increase in temperature, the reading at $t°$ would be $-[42.66° - (0.5t)]$.

At 20°C., for example, the reading would be − 32.66°. Since the change in rotation has been from + 100° to $-[42.66° - 0.5t]$, or $142.66 - 0.5t$, this value may be used as a measure of the amount of sucrose present; provided, of course, that this change in rotation is due to the sucrose alone.

Procedure.—(*a*) (*Herzfeld Method*).—Free the clarified solution used for direct polarization from lead by cautiously adding successive small portions of powdered potassium oxalate until no more lead is precipitated. Filter through a dry filter. Measure 50 cc. of the filtrate into a 100-cc. flask, add 25 cc. of water and add gradually, mixing thoroughly, 5 cc. of concentrated hydrochloric acid (sp. gr. 1.19). Place a thermometer in the flask so that its bulb is in the center of the solution and immerse the flask in a water-bath of suitable temperature. The temperature of

[1] W. D. Horne: *J. Am. Chem. Soc.*, **1904,** 186; **1907,** 926.

the solution should reach 69°C. in 2.5 to 3 minutes and is to be kept between 68° and 70° for 7 to 7.5 minutes, making a total time of heating of 10 minutes. It will probably be found best to provide two baths, one kept at 70° and the other at about 85°C. in which the flask may be immersed at first and then transferred to the 70° bath when the temperature approaches 66° to 67°. At the end of the 10 minutes heating, cool the solution quickly to 20° C., rinse off the thermometer into the flask and make up the volume to 100 cc. Mix thoroughly, filter if necessary, and polarize in a tube provided with a thermometer by which the exact temperature at the time of polarizing may be noted. It is preferable, as in the direct polarization, to make the reading at 20°C. The reading, corrected for zero error, as before, and multiplied by two, is the "invert polarization," of the sample.

The sucrose is calculated by the formula:

$$S = \frac{100\,(P - I)}{142.66 - 0.5t}$$

where S = percentage of sucrose, P the direct polarization, I the invert polarization and t the temperature at which the invert polarization was made. Note that $(P - I)$ means the *algebraic difference*, which in actual figures may be the *sum* of the two readings.

(*b*) *Modified Method at Room Temperature.*—If the longer time required is not objectionable, the inversion with hydrochloric acid may be carried out at room temperature, thereby avoiding the danger of partial destruction of levulose by too long or too high heating.

Place 50 cc. of the solution, freed from lead as directed above, in a 100-cc. graduated flask, add 5 cc. of concentrated hydrochloric acid and allow the flask to stand at 20°–25°C. (room temperature) for 20–24 hours, make up to 100 cc. and polarize as before. Sucrose is calculated by the Herzfeld formula given above.

Notes.—The methods as described should be carefully followed, especially as regards the time and temperature of inversion. Levulose is easily decomposed during inversion and its optical rotation is greatly affected by slight changes of temperature.

The temperature during inversion should not vary more than 1° on either side of 69°C. and must not in any case go above 70°C.

The Herzfeld formula as given is correct only for a solution containing half the normal weight in 100 cc. which would be the case if the normal weight were taken originally. The inversion factor varies from 141.85 for 1 gram of sucrose in 100 cc. to 143.54 for the normal weight. More accurate results can be obtained by the general formula suggested by Herzfeld.[1]

$$S = \frac{100\,(P - P')}{141.84 + 0.05N - 0.5t}$$

where S = per cent. of sucrose, P and P' are the direct and invert polarizations, respectively, for a normal weight and N is the scale reading of the inverted solution.

It is also true, of course, that factors worked out for pure sucrose solutions are not absolutely exact for the less pure food products.

The effect of the hydrochloric acid present during polarization on the specific rotation of levulose, should also be borne in mind in some cases. In the polarization of jams or honey, for example, an error is caused by the presence of considerable quantities of invert sugar, which would have a different rotation in the direct polarization in neutral solution than in the invert polarization in the presence of hydrochloric acid.

In some cases, where it is especially desirable to avoid the complications arising from the use of acid, inversion may be accomplished by the use of invertase, prepared from yeast. This method is more troublesome to carry out and takes longer, but gives better results. Detailed directions for preparing a stock invertase solution and for carrying out the test are given by Hudson.[2]

Invariably where the double polarization method is employed and it is necessary to clarify the solution by basic lead acetate, the clarification should be done before the direct polarization, otherwise the invert polarization will be too low on account of the retention of a portion of the invert sugar by the lead precipitate. The excess of lead should be removed, as directed on

[1] *Z. Ver. Deut. Zuckerind.*, **1890,** 194.
[2] *J. Ind. Eng. Chem.*, **1910,** 143.

page 257, otherwise the concentration of the hydrochloric acid will be altered by the precipitation of lead chloride.

With food products in general, the accuracy of the method is not greater than 0.5 per cent. With pure sugars, of course, the accuracy is much greater.

Calculation of Other Sugars from the Double Polarization.—If only one other sugar is present besides the sucrose, its amount may be calculated by the formula:

$$X = \frac{66.5\ (P - S)}{sp}$$

where P is the direct polarization (for the normal weight), S the percentage of sucrose as calculated from the double polarization, 66.5 the specific rotation at 20°C. of sucrose, and sp the specific rotation at 20°C. of the second sugar.[1]

Example.—A sample of jelly, in which sucrose and invert sugar are present, polarizes directly + 47.0°, and after inversion polarizes − 20.1° at 20°C. What is the percentage of sucrose and of invert sugar?

$$\text{Sucrose} = \frac{100\ [47.0 - (-20.1)]}{142.66 - \frac{20}{2}} = 50.58 \text{ per cent.}$$

$$\text{Invert sugar} = \frac{66.5\ (47.0 - 50.58)}{-20} = 11.91 \text{ per cent.}$$

Note.—With most food products, the result obtained in this way is not strictly exact on account of the errors inherent in the sucrose determination, as well as the error caused by the occlusion of reducing sugars in the basic lead precipitate.

Polarization at 87°C.—Considerable use is made in food analysis of polarization at higher temperatures than 20°C., especially of polarization of the inverted solution at 87°C. in the approximate determination of commercial glucose.

Solutions of invert sugar become optically inactive when heated to a temperature of about 87°C. This is on account of the decrease in specific rotation of levulose with increasing temperature until it just equals that of the dextro-rotatory dextrose. The exact temperature of neutralization varies somewhat

[1] See table on page 247.

with the concentration, but 87°C. is the value ordinarily used. If we take the case of a table sirup in which the sugars present are sucrose, invert sugar and commerical glucose, by polarizing the inverted solution at 87° the commercial glucose will be the only optically active sugar of the three, and can thus be determined from the polariscopic reading. Certain exceptions to this statement will be noted later.

The saccharimeter ordinarily employed may be used to make the readings at 87°, but on account of the considerable difference in coefficient of expansion between glass and metal, and the consequent difficulty of securing a tight joint with successive heating and cooling, an all-metal, jacketed tube is best for the solution. Care should be taken that the inner tube is not of too small bore, otherwise the bubbles formed during the heating are apt to prove troublesome. The hot water for the jacket may be supplied from a large tank of boiling water placed at a little distance from the instrument and high enough so that the hot water may flow by gravity through a rubber tube, the temperature being controlled by regulating the flow with a stopcock. Sy[1] has devised a simple and practical plan for this purpose. Great care should be taken to avoid heating the instrument. It is better to heat the tube while outside of the trough and place it in position only when nearly at the desired temperature.

Both on account of employing a metal tube and to avoid any action of the acid on the commercial glucose during the heating, it is best to polarize a separate neutral solution as follows:

Procedure.—Weigh out the normal weight of sample and invert it as described on page 257. To the inverted solution in a 100-cc. flask add a few drops of phenolphthalein and strong sodium hydroxide solution until slightly pink; add a drop of dilute hydrochloric acid in excess, make up to the mark and filter, if necessary. Fill the jacketed tube and heat fairly rapidly to about 80°C. Place the tube in the trough of the saccharimeter, first examining it to see that no air-bubbles are present, and heat steadily to 87°. Take the average of five or six rapid readings at this point, keeping the tube in the instrument no longer than is absolutely necessary. The slower heating when nearing the desired temperature is advisable in order to avoid troublesome

[1] *J. Am. Chem. Soc.*, **1908**, 1790.

convection currents. It is usually best to heat to several degrees above 87°, not above 90°, and take the readings when the solution *cools* to 87° rather than to read it while the solution is heating. Several series of observations should be made on successive fillings of the tube rather than spend time trying to find an exact end-point, on account of the greater difficulty usually experienced in reading. Readings can usually be taken between 86° and 88°.

Calculation of Commercial Glucose.—On account of the variable composition of commercial glucose, its specific rotation ranging from 130° to 154° for the anhydrous substance, it is obviously impossible to calculate the amount exactly from the saccharimeter reading. It is therefore customary to report the percentage in terms of some arbitrary standard. In the official methods of the Association of Official Agricultural Chemists,[1] it is assumed that the normal weight of commercial glucose polarizes + 175°. The action of the acid during inversion and the expansion of the solution at 87° reduce this value to + 163°.[2] Hence the calculation may be made by the formula:

$$G = \frac{100\,S}{163}$$

where G is the percentage of glucose and S the reading of the saccharimeter at 87°. It should be stated in the report that the factor 163 was used in the calculation. As noted more fully under Honey, page 296, the determination of commercial glucose by this method in such products as molasses, sirups and honey, is only approximate, because these materials usually contain dextrins and gums which render them slightly dextro-rotatory after inversion, even in the absence of commercial glucose.

Carbohydrates Other than Sugars

Determination of Starch.—In the case of nearly pure starch, it can be determined by hydrolysis with acid, as represented by the equation:

$$\underset{\text{Starch}}{\overset{(162)}{C_6H_{10}O_5}} + H_2O \rightarrow \underset{\text{Dextrose}}{\overset{(180)}{C_6H_{12}O_6}}$$

[1] *Bur. of Chem., Bull.* **107,** p. 71.

[2] Leach: *Bur. of Chem., Bull.* **81,** p. 74.

100 parts of dextrose correspond to 90 parts of starch. In most vegetable food products, however, the starch is accompanied by pentosans and other hemi-celluloses which yield reducing sugars upon hydrolysis, so that if exact results are desired the diastase method should be employed. The method of direct acid hydrolysis has the advantage of being much quicker and easier of execution and is often sufficiently accurate for the purpose.

Direct Acid Hydrolysis.—Weigh out from 2 to 5 grams of the sample, depending upon the amount of starch present, and if much fat is present, wash on a good quality filter paper with five successive portions of 10 cc. each of ether. Allow the ether to evaporate from the residue and then wash it with 10 per cent. alcohol to free it from soluble carbohydrates. Test the final washings by evaporation on the water-bath and note the presence of an appreciable residue. One hundred fifty cubic centimeters of alcohol will usually be enough for washing. Transfer the residue to a 500-cc. graduated flask with 200 cc. of water, add 20 cc. of hydrochloric acid (sp. gr. 1.125), place a funnel in the neck of the flask to prevent evaporation, and heat in a boiling water-bath for 2½ hours. Cool, nearly neutralize with sodium hydroxide, and make up to 500 cc. Filter and determine the dextrose in an aliquot part of the filtrate, using the Munson and Walker method, as described on page 237. The weight of dextrose multiplied by 0.90 gives the weight of starch.

Determination with Diastase.—The starch may be separated from the pentosans, which cause the high results of the preceding method, by digestion with diastase, which converts the starch into the soluble products, dextrin and maltose.

Procedure.—Treat 2 to 5 grams of the sample with ether and dilute alcohol as in the preceding method. Transfer the residue with 50 cc. of water to a beaker, heat slowly to boiling or immerse the beaker in boiling water until the starch gelatinizes, stirring constantly to avoid the formation of lumps. Fifteen minutes heating is usually sufficient. Cool to 55°C., add 20 cc. of malt extract[1] and keep the solution within 2° of this tem-

[1] To prepare an active malt extract, digest 10 grams of fresh, finely ground malt several hours at room temperature with 200 cc. of water, shaking occasionally, and filter. Add a few drops of chloroform to prevent the growth of molds.

perature for an hour. Heat again to boiling to gelatinize any remaining starch granules. Cool to 55°C., add 20 cc. of malt extract and maintain at this temperature for an hour, or until a drop of the solution, carefully examined under the microscope, fails to give the iodine reaction for starch. Cool, make up to 250 cc., and filter. Transfer 200 cc. of the filtrate to a 500-cc. graduated flask, add 20 cc. of hydrochloric acid (sp. gr. 1.125), and carry out the hydrolysis as described in the preceding method.

A blank determination must be carried through in precisely the same manner, using 50 cc. of water and the same amount of malt extract as in the regular procedure, in order to correct for the cupric reducing power of the malt extract itself.

Notes.—The method of determination by diastase gives results which are, in general, lower than those by direct hydrolysis with acid. In food materials which are comparatively high in starch, as ordinary cereals, the difference may be only 3 or 4 per cent.; in materials low in starch content but containing large amounts of pentosans, as cocoa shells, or mustard hulls, the results by acid hydrolysis may be ten to twenty times too high.

Although the factor 0.90 for the calculation of starch from dextrose has been adopted by the Association of Official Agricultural Chemists[1] as standard, it has been shown by various chemists that this theoretical value is never obtained, the actual result being from 95 to 98 per cent. of theory, so that more exact results are probably secured by using a somewhat higher factor (0.92 to 0.94) to calculate the starch.

Determination of Pentosans.—The pentosans may be determined by hydrolyzing with dilute acid to the corresponding pentoses and estimating the latter with Fehling's solution,[2] the entire method being analogous to the determination of starch by acid hydrolysis. In complex food materials, however, which contain also other carbohydrates which either reduce Fehling's solution directly or are hydrolyzed to such reducing substances, a better method is to heat the pentosans with hydrochloric acid under such conditions that the pentoses formed by hydrolysis shall split up into furfural and water. The method is an elaboration of the

[1] *Bur. of Chem., Bull.* **107,** p. 54.

[2] Stone: *J. Am. Chem. Soc.,* **1897,** 183.

qualitative test described on page 235, and although the reaction does not yield the theoretical quantity of furfural, the conditions may be so standardized that the results are practically quantitative.

The furfural which is formed may be determined by weighing the insoluble condensation product which it forms with phenylhydrazine:

$$\underset{\text{Furfural}}{C_4H_3O.CHO} + \underset{\text{Phenylhydrazine}}{C_6H_5 - NH - NH_2} \rightarrow \underset{\text{Furfural-phenylhydrazone}}{C_4H_3.CH = N_2H.C_6H_5} + H_2O$$

or with phloroglucinol.[1] The latter method has been adopted by the Association of Official Agricultural Chemists[2] and is the one generally employed in this country.

Method.—(*a*) *Distillation.*—Place 2 to 5 grams of the material, according to the amount of pentosan present, in a 300-cc. distilling flask with 100 cc. of 12 per cent. hydrochloric acid (sp. gr. 1.06) and several bits of recently heated pumice. Close the flask with a rubber stopper carrying a 50-cc. separatory funnel. Place the flask on wire gauze, connect with a condenser, and heat, rather gently at first, then so regulate the flame as to distil over 30 cc. in about 10 minutes. Collect the distillate in a graduated receiver, passing it through a small filter paper as it distils. Replace the 30 cc. distilled over by 30 cc. more of the dilute hydrochloric acid, adding it through the separatory funnel in such a manner as to wash down the particles adhering to the sides of the flask and continue the process in similar manner until the distillate amounts to 360 cc.

(*b*) *Precipitation.*—To the completed distillate add slowly a measured quantity of phloroglucinol (see below) dissolved in 12 per cent. hydrochloric acid. The amount of phloroglucinol added should be about double that of the furfural expected. The solution first turns yellow, then green, and then an amorphous greenish precipitate appears, which grows rapidly darker, till it finally becomes almost black. Make the solution up to 400 cc. with 12 per cent. hydrochloric acid and allow it to stand over night. Test the solution with anilin acetate paper (see page 235) to make sure that all the furfural has been precipitated,

[1] Councler: *Chem.-Ztg.*, **1893**, 743.

[2] *Bur. of Chem., Bull.* **107**, p. 54.

and filter through a weighed Gooch crucible; the precipitate of phloroglucide is brought carefully upon the asbestos and washed with 150 cc. of water in such a way that the water is not entirely removed from the crucible until the very last. Place the crucible upon a triangle or suitable support, so that the bottom is free to the air, and dry for 4 hours at the temperature of boiling water; place in a weighing bottle, cool in a desiccator and weigh.

Calculation.—The composition of the furfural-phloroglucide varies somewhat according to the proportion of furfural present, hence the percentage of furfural, and consequently of pentosans, cannot be calculated exactly by a factor. Krober[1] has tabulated the values of furfural, pentoses and pentosans for weights of phloroglucide lying between 0.030 and 0.300 gram and a condensed statement of his table is given on page 267. The value given in the column headed "Pentosan" is in each case the mean of the corresponding values for xylan and araban.

For weights of phloroglucide below 0.030 gram the weight of pentose may be found from the weight of phloroglucide (a) by the formula:

$$\text{Pentose} = (a \text{ plus } 0.0052) \times 1.0170$$

and for weights over 0.300 gram

$$\text{Pentose} = (a \text{ plus } 0.0052) \times 1.0026$$

The corresponding amount of pentosan may be obtained by multiplying the pentose by 0.88.

$$\text{Pentose : pentosan} = (C_5H_{10}O_5)_n : (C_5H_8O_4)_n = 150 : 132 = 1.0 : 0.88$$

The value 0.0052 is the weight in grams of phloroglucide which remains dissolved in the 400 cc. of solution.

It will be found best to use such a quantity of material that the weight of phloroglucide shall fall between 0.030 and 0.300 gram.

Notes.—Care should be taken during the distillation that charring of the material which may collect on the sides of the flask above the liquid does not occur, since furfural might be formed by the decomposition. For this reason the heating

[1] *J. Landw.*, **1900**, 355; **1901**, 7.

TABLE XXXV.—KROBER'S TABLE FOR DETERMINING PENTOSES AND PENTOSANS

Furfural-phloroglucide	Furfural	Arabinose	Araban	Xylose	Xylan	Pentose	Pentosan
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
0.035	0.0209	0.0446	0.0393	0.0370	0.0326	0.0408	0.0359
0.040	0.0235	0.0501	0.0441	0.0416	0.0366	0.0459	0.0404
0.045	0.0260	0.0556	0.0490	0.0462	0.0406	0.0509	0.0448
0.050	0.0286	0.0611	0.0538	0.0507	0.0446	0.0559	0.0492
0.055	0.0312	0.0666	0.0586	0.0553	0.0486	0.0610	0.0537
0.060	0.0338	0.0721	0.0634	0.0598	0.0526	0.0660	0.0581
0.065	0.0364	0.0776	0.0683	0.0644	0.0567	0.0710	0.0625
0.070	0.0390	0.0831	0.0731	0.0690	0.0607	0.0761	0.0670
0.075	0.0416	0.0886	0.0780	0.0736	0.0647	0.0811	0.0714
0.080	0.0442	0.0941	0.0828	0.0781	0.0687	0.0861	0.0758
0.085	0.0468	0.0996	0.0877	0.0827	0.0727	0.0912	0.0803
0.090	0.0494	0.1051	0.0925	0.0872	0.0767	0.0962	0.0847
0.095	0.0520	0.1106	0.0974	0.0918	0.0808	0.1012	0.0891
0.100	0.0546	0.1161	0.1022	0.0964	0.0848	0.1063	0.0935
0.105	0.0572	0.1215	0.1070	0.1010	0.0888	0.1113	0.0979
0.110	0.0598	0.1270	0.1118	0.1055	0.0928	0.1163	0.1023
0.115	0.0624	0.1325	0.1166	0.1101	0.0968	0.1213	0.1067
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
0.125	0.0676	0.1435	0.1263	0.1192	0.1049	0.1314	0.1156
0.130	0.0702	0.1490	0.1311	0.1237	0.1089	0.1364	0.1201
0.135	0.0728	0.1545	0.1360	0.1283	0.1129	0.1414	0.1244
0.140	0.0754	0.1600	0.1408	0.1328	0.1169	0.1464	0.1288
0.145	0.0780	0.1655	0.1457	0.1378	0.1209	0.1515	0.1333
0.150	0.0805	0.1710	0.1505	0.1419	0.1249	0.1565	0.1377
0.155	0.0831	0.1765	0.1554	0.1465	0.1289	0.1615	0.1421
0.160	0.0857	0.1820	0.1602	0.1510	0.1329	0.1665	0.1465
0.165	0.0883	0.1875	0.1650	0.1556	0.1369	0.1716	0.1510
0.170	0.0909	0.1930	0.1698	0.1601	0.1409	0.1766	0.1554
0.175	0.0935	0.1985	0.1746	0.1647	0.1449	0.1816	0.1598
0.180	0.0961	0.2039	0.1794	0.1692	0.1489	0.1866	0.1642
0.185	0.0987	0.2093	0.1842	0.1738	0.1529	0.1916	0.1686
0.190	0.1013	0.2147	0.1889	0.1783	0.1569	0.1965	0.1729
0.195	0.1039	0.2201	0.1937	0.1829	0.1609	0.2015	0.1773
0.200	0.1065	0.2255	0.1984	0.1874	0.1649	0.2065	0.1817
0.205	0.1090	0.2309	0.2032	0.1920	0.1689	0.2115	0.1861
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0 2164	0.1904
0.215	0.1142	0.2417	0.2127	0.2011	0.1770	0.2214	0.1948
0.220	0.1168	0.2471	0.2174	0.2057	0.1810	0.2264	0.1992
0.225	0.1194	0.2525	0.2222	0.2102	0.1850	0.2314	0.2037

TABLE XXXV.—KROBER'S TABLE FOR DETERMINING PENTOSES AND PENTOSANS.—(*Continued*)

Furfural-phloroglucide	Furfural	Arab-inose	Araban	Xylose	Xylan	Pentose	Pento-san
0.230	0.1220	0.2579	0.2270	0.2148	0.1890	0.2364	0.2081
0.235	0.1245	0.2633	0.2318	0.2193	0.1930	0.2413	0.2124
0.240	0.1271	0.2687	0.2365	0.2239	0.1970	0.2463	0.2168
0.245	0.1297	0.2741	0.2413	0.2284	0.2010	0.2513	0.2212
0.250	0.1323	0.2795	0.2460	0.2330	0.2050	0.2563	0.2256
0.255	0.1349	0.2849	0.2508	0.2375	0.2090	0.2612	0.2299
0.260	0.1374	0.2903	0.2555	0.2420	0.2130	0.2662	0.2342
0.265	0.1400	0.2957	0.2603	0.2465	0.2170	0.2711	0.2385
0.270	0.1426	0.3011	0.2650	0.2511	0.2210	0.2761	0.2429
0.275	0.1452	0.3065	0.2698	0.2556	0.2250	0.2811	0.2473
0.280	0.1478	0.3199	0.2745	0.2602	0.2290	0.2861	0.2517
0.285	0.1504	0.3173	0.2793	0.2647	0.2330	0.2910	0.2561
0.290	0.1529	0.3227	0.2840	0.2693	0.2370	0.2960	0.2605
0.295	0.1555	0.3281	0.2887	0.2738	0.2410	0.3010	0 2649
0.300	0.1581	0.3335	0.2935	0.2784	0.2450	0.3060	0.2693

should be done carefully and the material washed down as much as possible when adding the acid. The method contains two inherent sources of error in that pentosans are not the only substances present in food products which yield furfural upon heating with hydrochloric acid, and also that insoluble compounds with phloroglucinol may be formed by other products of the acid distillation than furfural.

Cellulose, starch and the sugars ordinarily found in foods, when distilled with hydrochloric acid will give a slight precipitate with phloroglucinol, so that if very small amounts of phloroglucide are obtained the possibility of its coming from this source should not be overlooked.

Methyl-furfural, obtained by distilling methyl-pentoses with hydrochloric acid, and oxymethyl-furfural, which may be produced in small amounts by the action of hydrochloric acid on sucrose or levulose, will both precipitate with phloroglucinol. Various substances not furfural, the so-called "furaloids,"[1] may be present in the hydrochloric acid distillate and will precipitate with phloroglucinol. These, however, can be removed by re-distilling the acid distillate. The determination of pentosans,

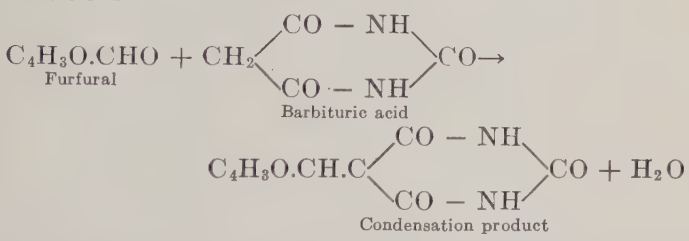
[1] Fraps: *Am. Chem. J.*, **1901**, 501.

even when carried out with great care, should by no means be considered as yielding results of great exactness.

It has been claimed by Jager and Unger[1] that barbituric acid is a better precipitant of furfural than phloroglucinol since it gives no precipitate with the distillates from the hexoses, as starch and sucrose. The method is as follows:

Treat the hydrochloric acid distillate, obtained as above, with a solution of pure barbituric acid in hydrochloric acid of 1.06 specific gravity, using eight times as much barbituric acid as the pentosan expected. Stir the mixture, allow to stand for 24 hours, filter the granular yellow precipitate in a Gooch crucible, wash with water and dry at 105°C. for 4 hours. Increase the weight of precipitate by 0.0049 gram for the amount that remains dissolved in the 400 cc. of acid solution.

The reaction is



From the equation may be readily calculated the weight of furfural corresponding to the weight of the condensation product obtained, and from this the pentosans.

The method has not as yet come into such common use as the older phloroglucinol method.

Determination of Crude Fiber.—By the term "crude fiber" is ordinarily meant in agricultural and food analysis the residue, consisting largely of cellulose, which is left after the other carbohydrates and the proteins have been removed by successive treatments with boiling acid and alkali. The method which has been adopted by the Association of Official Agricultural Chemists as official is the one devised by Henneberg, and usually called the "Weende" method from the experiment st tion of that name near Göttingen, where it was originally employed.

Method.—If the material contains an appreciable amount of

[1] *Ber.*, **1902,** 4440; **1903,** 1222.

fat, extract about 2 grams of it with ordinary ether, or use the residue from the determination of "ether extract." Transfer the extracted material to a 500-cc. Erlenmeyer flask, add 200 cc. of boiling 1.25 per cent. sulphuric acid and heat at once to boiling, keeping a small funnel in the neck of the flask to prevent evaporation. Boil gently for 30 minutes, whirling the flask cautiously from time to time to rinse down material which may have collected on its sides. Filter through a ribbed filter-paper and wash once with boiling water. Heat 200 cc. of 1.25 per cent. sodium hydroxide, free from carbonate, to boiling in a small wash bottle and with it rinse the substance from the filter through a large funnel back into the Erlenmeyer flask. Boil at once and continue the gentle boiling for 30 minutes as before. Filter on a Gooch crucible, keeping the residue from the filter until the last, and wash with boiling water until free from alkali. Reject the hot filtrate from the filter bottle, wash the crucible once with alcohol and once with ether and dry at 100°C. to constant weight. Ignite, cautiously at first, then at a low red heat until completely incinerated, and weigh again. The loss in weight is the crude fiber.

Notes.—The material should be as finely ground as possible, since with coarse particles the results may be much too high. In general the substance should be ground so that it will all pass through a sieve of 0.5-mm. mesh or at least through a sieve with round holes 1 mm. in diameter. Some very finely divided substances, on the other hand, tend to clog the Gooch crucible during the final filtration, and should be washed as far as possible by decantation. With substances rich in proteins it may be found advantageous to boil with alkali first and then with acid. The strength of the acid and alkali should be carefully determined by titration.

The crude fiber obtained in this way is not pure cellulose, but contains distinct proportions of hemicelluloses,[1] pentosans and nitrogenous substances. These, however, are not sufficient to prevent the results from being reasonably accurate and comparable.

A crude fiber which is practically free from pentosans may be

[1] Tollens and Düring: *J. Landw.*, **1897,** 79; **1901,** 11.

obtained by the procedure of König[1] in which the substance is boiled with glycerin and sulphuric acid under pressure.

Three grams of the fat-free sample are boiled for an hour with 200 cc. of glycerin (sp. gr. 1.23) containing 4 grams of concentrated sulphuric acid. The boiling may be done in a flask, provided with a reflux condenser, at 133° to 135°C., or preferably in an autoclave at 137°C., corresponding to a pressure of 3 atmospheres. After cooling to 80°–100°, the solution is diluted with 200 to 250 cc. of boiling water and filtered hot through an asbestos filter. The residue is washed with hot water, then with alcohol and ether, dried to constant weight and ignited as in the Weende method.

Maple Sirup

Source and Methods of Manufacture.—Maple sirup is an essentially American product, being practically unknown to European countries and having a somewhat limited range on this continent. Its use dates back to the first white settlers in this country who, in all probability, learned its nature and source from the Indians.

It is usually prepared from the sap obtained by tapping the hard or rock maple tree (*Acer saccharum*). One or more holes about half an inch in diameter and 1 to 3 in. deep, according to the size of the tree, are bored. After cleaning the hole, a metal spout is driven in and from this is hung a bucket to catch the sap.

The composition of the sap varies during the season and with the conditions of weather and sunlight, but it is essentially a dilute solution of sucrose containing traces of invert sugar, malic acid, mineral matter and albuminoids. The average content of sucrose is 3 per cent., and the ordinary yield of sugar from a single tree during the season is about 3 pounds.

The sirup may be made from the sap by concentration in open kettles or by more modern continuous evaporating apparatus. A great many of the small producers still use the primitive kettle method, in which sap is boiled sometimes all day, fresh sap being added continually and the sirup removed at night. The product obtained by this long boiling is dark colored and quite impure, with a strong flavor. The larger

[1] *Z. Nahr. Genussm.*, **1898**, 1.

producers use continuous evaporators in which the sap enters at one end and passes gradually from one compartment to another, the sirup being drawn off at the other end. This more rapid concentration produces a lighter colored product. During the evaporation, the proteins are coagulated and skimmed off, and a small amount of insoluble residue or "niter," consisting mainly of calcium malate, is deposited. For making sirup the sap is boiled until the sirup weighs 11 pounds to the gallon, corresponding to a specific gravity of 1.325. If thinner than this the sirup is liable to ferment; if the density is above 1.325, sugar will crystallize from the sirup.

Strictly speaking, maple sirup made in this way should be called maple-sap sirup to distinguish it from maple-sugar sirup, made by dissolving the solid product of further evaporation in water.

Forms of Adulteration.—The most common adulterant of maple products is refined or granulated sugar, added as such to the maple sugar, and in the form of sirup to maple sirup. Brown sugar, refinery sirup, or even molasses may be occasionally used, but these adulterants have too pronounced a flavor to permit their use in large amount. Commercial glucose, which was formerly quite extensively used as an adulterant, is not commonly employed now. Preservatives, especially sodium benzoate, may be present occasionally.

Doolittle and Seeker[1] have pointed out a possible adulterant of maple products in Muscovado sugar, a brown-colored tropical raw sugar. The following table shows the great similarity between this product and genuine maple sugar:

TABLE XXXVI. -COMPARISON OF MUSCOVADO AND MAPLE SUGARS

Determination	Light Muscovado sugar	Dark Muscovado sugar	Vermont maple sugar
Moisture (per cent.)	7.35	7.50	2.80
Ash (per cent.)	1.33	1.30	1.10
Polarization, direct (°V.)	+80.0	+82.4	+84.0
Polarization, invert (°V.)	−27.0	−26.8	−29.6
Polarization, invert at 86° (°V.)	0.0	0.0	0.0
Sucrose (per cent.)	81.4	83.1	85.6
Winton lead number	2.08	2.12	2.26

[1] *Bur. of Chem., Bull.* **122,** p. 196.

As shown on page 290, however, the composition of the ash is sufficiently different from that of maple sugar to serve to detect the product.

Methods of Analysis[1]

Moisture.—As with saccharine products in general, three methods are available for determining the water content.

(*a*) *Direct Drying.*—Spread about 10 to 15 grams of clean, ignited quartz sand in a flat-bottom platinum dish, add a short stirring rod, and weigh. Add 2 to 5 grams of the sirup and enough water so that it may be thoroughly mixed with the sand. Dry on the water-bath with frequent stirring and finally for 7 to 8 hours in the oven at 100°C., or until the loss in weight during 1 hour does not exceed 3 mgm.

Note.—The method described, although tedious as compared with those mentioned below, gives results of reasonable accuracy with maple sirup. With food products, however, containing notable amounts of levulose, as honey, molasses and jams, the results are always too low on account of the partial decomposition of that sugar. (See also page 437.) With such products the drying should be done in a vacuum oven at a temperature not exceeding 70°C., for which reason it is usually more convenient to employ one of the other methods.

(*b*) *From Specific Gravity.*—Weigh out 20 grams of the sirup, conveniently in a sugar dish, page 255, and transfer to a 100-cc. flask. Dissolve in water and make up to the mark. Determine the specific gravity $\left(\frac{20^\circ}{4^\circ}\text{ C.}\right)$ of the solution by a pyknometer and note the corresponding percentage of solids from Table XXXVII, page 274. Calculate the solids in the original sirup by the formula

$$P = \frac{VDS}{W}$$

where P = per cent. of solids in the undiluted sample; V = volume of diluted solution; D = specific gravity of diluted solution;

[1] The methods of analysis given here, although described mainly for maple sirup, can be applied equally well to maple sugar by using a corresponding weight, or in some cases more simply by dissolving a weighed amount of sugar in water, making up to definite volume and using aliquots for the various determinations.

TABLE XXXVII.—SPECIFIC GRAVITY[1] OF SOLUTIONS OF CANE SUGAR AT $\frac{20°}{4°}$ C.

Per cent. sugar	Tenths of per cent.									
	0	1	2	3	4	5	6	7	8	9
0	0.998234	0.998622	0.999010	0.999398	0.999786	1.000174	1.000563	1.000952	1.001342	1.001
1	1.002120	1.002509	1.002897	1.003286	1.003675	1.004064	1.004453	1.004844	1.005234	1.005
2	1.006015	1.006405	1.006796	1.007188	1.007580	1.007972	1.008363	1.008755	1.009148	1.009
3	1.009934	1.010327	1.010721	1.011115	1.011510	1.011904	1.012298	1.012694	1.013089	1.013
4	1.013881	1.014277	1.014673	1.015070	1.015467	1.015864	1.016261	1.016659	1.017058	1.017
5	1.017854	1.018253	1.018652	1.019052	1.019451	1.019851	1.020251	1.020651	1.021053	1.021
6	1.021855	1.022257	1.022659	1.023061	1.023463	1.023867	1.024270	1.024673	1.025077	1.025
7	1.025885	1.026289	1.026694	1.027099	1.027504	1.027910	1.028316	1.028722	1.029128	1.029
8	1.029942	1.030349	1.030757	1.031165	1.031573	1.031982	1.032391	1.032800	1.033209	1.033
9	1.034029	1.034439	1.034850	1.035260	1.035671	1.036082	1.036494	1.036906	1.037318	1.037
10	1.038143	1.038556	1.038970	1.039383	1.039797	1.040212	1.040626	1.041041	1.041456	1.041
11	1.042288	1.042704	1.043121	1.043537	1.043954	1.044370	1.044788	1.045206	1.045625	1.046
12	1.046462	1.046881	1.047300	1.047720	1.048140	1.048559	1.048980	1.049401	1.049822	1.050
13	1.050665	1.051087	1.051510	1.051933	1.052356	1.052778	1.053202	1.053626	1.054050	1.054
14	1.054900	1.055325	1.055751	1.056176	1.056602	1.057029	1.057455	1.057882	1.058310	1.058
15	1.059165	1.059593	1.060022	1.060451	1.060880	1.061308	1.061738	1.062168	1.062598	1.063
16	1.063460	1.063892	1.064324	1.064756	1.065188	1.065621	1.066054	1.066487	1.066921	1.067
17	1.067789	1.068223	1.068658	1.069093	1.069529	1.069964	1.070400	1.070836	1.071273	1.071
18	1.072147	1.072585	1.073023	1.073461	1.073900	1.074338	1.074777	1.075217	1.075657	1.076
19	1.076537	1.076978	1.077419	1.077860	1.078302	1.078744	1.079187	1.079629	1.080072	1.080
20	1.080959	1.081403	1.081848	1.082292	1.082737	1.083182	1.083628	1.084074	1.084520	1.084
21	1.085414	1.085861	1.086309	1.086757	1.087205	1.087652	1.088101	1.088550	1.089000	1.089
22	1.089900	1.090351	1.090802	1.091253	1.091704	1.092155	1.092607	1.093060	1.093513	1.093
23	1.094420	1.094874	1.095328	1.095782	1.096236	1.096691	1.097147	1.097603	1.098058	1.098
24	1.098971	1.099428	1.099886	1.100344	1.100802	1.101259	1.101718	1.102177	1.102637	1.103
25	1.103557	1.104017	1.104478	1.104938	1.105400	1.105862	1.106324	1.106786	1.107248	1.107
26	1.108175	1.108639	1.109103	1.109568	1.110033	1.110497	1.110963	1.111429	1.111895	1.112
27	1.112828	1.113295	1.113763	1.114229	1.114697	1.115166	1.115635	1.116104	1.116572	1.117
28	1.117512	1.117982	1.118453	1.118923	1.119395	1.119867	1.120339	1.120812	1.121284	1.121
29	1.122231	1.122705	1.123179	1.123653	1.124128	1.124603	1.125079	1.125555	1.126030	1.126
30	1.126984	1.127461	1.127939	1.128417	1.128896	1.129374	1.129853	1.130332	1.130812	1.131
31	1.131773	1.132254	1.132735	1.133216	1.133698	1.134180	1.134663	1.135146	1.135628	1.136
32	1.136596	1.137080	1.137565	1.138049	1.138534	1.139020	1.139506	1.139993	1.140479	1.140
33	1.141453	1.141941	1.142429	1.142916	1.143405	1.143894	1.144384	1.144874	1.145363	1.145
34	1.146345	1.146836	1.147328	1.147820	1.148313	1.148805	1.149298	1.149792	1.150286	1.150
35	1.151275	1.151770	1.152265	1.152760	1.153256	1.153752	1.154249	1.154746	1.155242	1.155
36	1.156238	1.156736	1.157235	1.157733	1.158233	1.158733	1.159233	1.159733	1.160233	1.160
37	1.161236	1.161738	1.162240	1.162742	1.163245	1.163748	1.164252	1.164756	1.165259	1.165
38	1.166269	1.166775	1.167281	1.167786	1.168293	1.168800	1.169307	1.169815	1.170322	1.170
39	1.171340	1.171849	1.172359	1.172869	1.173379	1.173889	1.174400	1.174911	1.175423	1.175

[1] According to Dr. F. Plato (Wiss. Abh. der Kaiserlichen Normal-Eichungs-Kommission, p. 153; 1900).

TABLE XXXVII.—SPECIFIC GRAVITY OF SOLUTIONS OF CANE SUGAR AT $\frac{20°}{4°}$ C.

—(*Continued*)

Tenths of per cent.									
0	1	2	3	4	5	6	7	8	9
1.176447	1.176960	1.177473	1.177987	1.178501	1.179014	1.179527	1.180044	1.180560	1.181076
1.181592	1.182108	1.182625	1.183142	1.183660	1.184178	1.184696	1.185215	1.185734	1.186253
1.186773	1.187293	1.187814	1.188335	1.188856	1.189379	1.189901	1.190423	1.190946	1.191469
1.191993	1.192517	1.193041	1.193565	1.194090	1.194616	1.195141	1.195667	1.196193	1.196720
1.197247	1.197775	1.198303	1.198832	1.199360	1.199890	1.200420	1.200950	1.201480	1.202010
1.202540	1.203071	1.203603	1.204136	1.204668	1.205200	1.205733	1.206266	1.206801	1.207335
1.207870	1.208405	1.208940	1.209477	1.210013	1.210549	1.211086	1.211623	1.212162	1.212700
1 213238	1.213777	1.214317	1.214856	1.215395	1.215936	1.216476	1.217017	1.217559	1.218101
1.218643	1.219185	1.219729	1.220272	1.220815	1.221360	1.221904	1.222449	1.222995	1.223540
1.224086	1.224632	1.225180	1.225727	1.226274	1.226823	1.227371	1.227919	1.228469	1.229018
1.229567	1.230117	1.230668	1.231219	1.231770	1.232322	1.232874	1.233426	1.233979	1.234532
1.235085	1.235639	1.236194	1.236748	1.237303	1.237859	1.238414	1.238970	1.239527	1.240084
1.240641	1.241198	1.241757	1.242315	1.242873	1.243433	1.243992	1.244552	1.245113	1.245673
1.246234	1.246795	1.247358	1.247920	1.248482	1.249046	1.249609	1.250172	1.250737	1.251301
1.251866	1.252431	1.252997	1.253563	1.254129	1.254697	1.255264	1.255831	1.256400	1.256967
1.257535	1.258104	1.258674	1.259244	1.259815	1.260385	1.260955	1.261527	1.262099	1.262671
1.263243	1.263816	1.264390	1.264963	1.265537	1.266112	1.266686	1.267261	1.267837	1.268413
1.268989	1.269565	1.270143	1.270720	1.271299	1.271877	1.272455	1.273035	1.273614	1.274194
1.274774	1.275354	1.275936	1.276517	1.277098	1.277680	1.278262	1.278844	1.279428	1.280011
1.280595	1.281179	1.281764	1.282349	1.282935	1.283521	1.284107	1.284694	1.285281	1.285869
1.286456	1.287044	1.287633	1.288222	1.288811	1.289401	1.289991	1.290581	1.291172	1.291763
1.292354	1.292946	1.293539	1.294131	1.294725	1.295318	1.295911	1.296506	1.297100	1.297696
1.298291	1.298886	1.299483	1.300079	1.300677	1.301274	1.301871	1.302470	1.303068	1.303668
1.304267	1.304867	1.305467	1.306068	1.306669	1.307271	1.307872	1.308475	1.309077	1.309680
1.310282	1.310885	1.311489	1.312093	1.312699	1.313304	1.313909	1.314515	1.315121	1.315728
1.316334	1.316941	1.317549	1.388157	1.318766	1.319374	1.319983	1.320593	1.321203	1.321814
1.322425	1.323036	1.323648	1.324259	1.324872	1.325484	1.326097	1.326711	1.327325	1.327940
1.328554	1.329170	1.329785	1.330401	1.331017	1.331633	1.332250	1.332868	1.333485	1.334103
1.334722	1.335342	1.335961	1.336581	1.337200	1.337821	1.338441	1.339063	1.339684	1.340306
1.340928	1.341551	1.342174	1.342798	1.343421	1.344046	1.344671	1.345296	1.345922	1.346547
1.347174	1.347801	1.348427	1.349055	1.349682	1.350311	1.350939	1.351568	1.352197	1.352827
1.353456	1.354087	1.354717	1.355349	1.355980	1.356612	1.357245	1.357877	1.358511	1.359144
1.359778	1.360413	1.361047	1.361682	1.362317	1.362953	1.363590	1.364226	1.364864	1.365501
1.366139	1.366777	1.367415	1.368054	1.368693	1.369333	1.369973	1.370613	1.371254	1.371894
1.372536	1.373178	1.373820	1.374463	1.375105	1.375749	1.376392	1.377036	1.377680	1.378326
1.378971	1.379617	1.380262	1.380909	1.381555	1.382203	1.382851	1.383499	1.384148	1.384796
1.385446	1.386096	1.386745	1.387396	1.388045	1.388696	1.389347	1.389999	1.390651	1.391303
1.391956	1.392610	1.393263	1.393917	1.394571	1.395226	1.395881	1.396536	1.397192	1.397848
1.398505	1.399162	1.399819	1.400477	1.401134	1.401793	1.402452	1.403111	1.403771	1.404430
1.405091	1.405752	1.406412	1.407074	1.407735	1.408398	1.409061	1.409723	1.410387	1.411051

TABLE XXXVII.—SPECIFIC GRAVITY OF SOLUTIONS OF CANE SUGAR AT $\frac{20°}{4°}$ C.

—(*Continued*)

Per cent. sugar	Tenths of per cent.									
	0	1	2	3	4	5	6	7	8	9
80	1.411715	1.412380	1.413044	1.413709	1.414374	1.415040	1.415706	1.416373	1.417039	1.417
81	1.418374	1.419043	1.419711	1.420380	1.421049	1.421719	1.422390	1.423059	1.423730	1.424
82	1.425072	1.425744	1.426416	1.427089	1.427761	1.428435	1.429109	1.429782	1.430457	1.431
83	1.431807	1.432483	1.433158	1.433835	1.434511	1.435188	1.435866	1.436543	1.437222	1.437
84	1.438579	1.439259	1.439938	1.440619	1.441299	1.441980	1.442661	1.443342	1.444024	1.444
85	1.445388	1.446071	1.446754	1.447438	1.448121	1.448806	1.449491	1.450175	1.450860	1.451
86	1.452232	1.452919	1.453605	1.454292	1.454980	1.455668	1.456357	1.457045	1.457735	1.458
87	1.459114	1.459805	1.460495	1.461186	1.461877	1.462568	1.463260	1.463953	1.464645	1.465
88	1.466032	1.466726	1.467420	1.468115	1.468810	1.469504	1.470200	1.470896	1.471592	1.472
89	1.472986	1.473684	1.474381	1.475080	1.475779	1.476477	1.477176	1.477876	1.478575	1.479
90	1.479976	1.480677	1.481378	1.482080	1.482782	1.483484	1.484187	1.484890	1.485593	1.486
91	1.487002	1.487707	1.488411	1.489117	1.489823	1.490528	1.491234	1.491941	1.492647	1.493
92	1.494063	1.494771	1.495479	1.496188	1.496897	1.497606	1.498316	1.499026	1.499736	1.500
93	1.501158	1.501870	1.502582	1.503293	1.504006	1.504719	1.505432	1.506146	1.506859	1.507
94	1.508289	1.509004	1.509720	1.510435	1.511151	1.511868	1.512585	1.513302	1.514019	1.514
95	1.515455	1.516174	1.516893	1.517612	1.518332	1.519051	1.519771	1.520492	1.521212	1.521
96	1.522656	1.523378	1.524100	1.524823	1.525546	1.526269	1.526993	1.527717	1.528441	1.529
97	1.529891	1.530616	1.531342	1.532068	1.532794	1.533521	1.534248	1.534976	1.535704	1.536
98	1.537161	1.537889	1.538618	1.539347	1.540076	1.540806	1.541536	1.542267	1.542998	1.543
99	1.544462	1.545194	1.545926	1.546659	1.547392	1.548127	1.548861	1.549595	1.550329	1.551
100	1.551800									

S = per cent. of solids from the table, and W = weight of sample taken. The moisture content is found by subtracting the total solids from 100.

If the specific gravity is taken at some other temperature than 20°C., the corresponding percentage of solids may be corrected by Table XXXVIII, on page 277. See also Table I, under General Methods, page 6, if it is desired to convert the specific gravity determined at $\frac{t°}{t°}$ to $\frac{t°}{4°}$.

Note.—It should be observed that the table on page 274 is based on the specific gravity of sucrose alone. It can be used for the determination of other pure sugars, since these do not differ greatly in specific gravity from equal concentrations of sucrose, but in the determination of total solids in impure products, containing a considerable proportion of non-sugars, the results

are not strictly accurate. In the case of such low-grade saccharine products as molasses, for instance, the determination by this method is only an approximation.

TABLE XXXVIII.—TEMPERATURE CORRECTIONS FOR CHANGING SUGAR PERCENTAGES TO CORRESPONDING VALUES AT 20°C.[1]

Temperature in degrees Centigrade	Observed per cent. of sugar													
	0	5	10	15	20	25	30	35	40	45	50	55	60	70
	Subtract from observed per cent.													
0.0	0.30	0.49	0.65	0.77	0.89	0.99	1.08	1.16	1.24	1.31	1.37	1.41	1.44	1.46
5.0	0.36	0.47	0.56	0.65	0.73	0.80	0.86	0.91	0.97	1.01	1.05	1.08	1.10	1.14
10.0	0.32	0.38	0.43	0.48	0.52	0.57	0.60	0.64	0.67	0.70	0.72	0.74	0.75	0.77
11.0	0.31	0.35	0.40	0.44	0.48	0.51	0.55	0.58	0.60	0.63	0.65	0.66	0.68	0.70
12.0	0.29	0.32	0.36	0.40	0.43	0.46	0.50	0.52	0.54	0.56	0.58	0.59	0.60	0.62
13.0	0.26	0.29	0.32	0.35	0.38	0.41	0.44	0.46	0.48	0.49	0.51	0.52	0.53	0.55
14.0	0.24	0.26	0.29	0.31	0.34	0.36	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47
15.0	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.33	0.34	0.36	0.36	0.37	0.38	0.39
16.0	0.17	0.18	0.20	0.22	0.23	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32
17.0	0.13	0.14	0.15	0.16	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24
18.0	0.09	0.10	0.10	0.11	0.12	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16
19.0	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08
17.5	0.11	0.12	0.12	0.14	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20
15.56 (60°F.)	0.18	0.20	0.22	0.24	0.26	0.28	0.29	0.30	0.30	0.32	0.33	0.33	0.34	0.34
	Add to observed per cent.													
21.0	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09
22.0	0.10	0.10	0.11	0.12	0.12	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.16
23.0	0.16	0.16	0.17	0.17	0.19	0.20	0.21	0.21	0.22	0.23	0.24	0.24	0.24	0.24
24.0	0.21	0.22	0.23	0.24	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.32	0.32
25.0	0.27	0.28	0.30	0.31	0.32	0.34	0.35	0.36	0.38	0.38	0.39	0.39	0.40	0.39
26.0	0.33	0.34	0.36	0.37	0.40	0.40	0.42	0.44	0.46	0.47	0.47	0.48	0.48	0.48
27.0	0.40	0.41	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.54	0.55	0.56	0.56	0.56
28.0	0.46	0.47	0.49	0.51	0.54	0.56	0.58	0.60	0.61	0.62	0.63	0.64	0.64	0.64
29.0	0.54	0.55	0.56	0.59	0.61	0.63	0.66	0.68	0.70	0.70	0.71	0.72	0.72	0.72
30.0	0.61	0.62	0.63	0.66	0.68	0.71	0.73	0.76	0.78	0.78	0.79	0.80	0.80	0.81
35.0	0.99	1.01	1.02	1.06	1.10	1.13	1.16	1.18	1.20	1.21	1.22	1.22	1.23	1.22
40.0	1.42	1.45	1.47	1.51	1.54	1.57	1.60	1.62	1.64	1.65	1.65	1.65	1.66	1.65
45.0	1.91	1.94	1.96	2.00	2.03	2.05	2.07	2.09	2.10	2.10	2.10	2.10	2.10	2.08
50.0	2.46	2.48	2.50	2.53	2.56	2.57	2.58	2.59	2.59	2.58	2.58	2.57	2.56	2.52
55.0	3.05	3.07	3.09	3.12	3.12	3.12	3.12	3.11	3.10	3.08	3.07	3.05	3.03	2.97
60.0	3.69	3.72	3.73	3.73	3.72	3.70	3.67	3.65	3.62	3.60	3.57	3.54	3.50	3.43
27.5	0.43	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.58	0.59	0.60	0.60	0.60

[1] *U. S. Bureau of Standards, Circ.* **19.**

(*c*) *By the Refractometer.*—Determine the refractive index of the sirup by the Abbe refractometer, as described on page 7, correcting, if necessary, for any deviation of temperature from 20°C. The average of three independent settings should be employed and unless the instrument is known to be in absolute adjustment it should be tested and any correction determined by readings on distilled water. The necessary data for calculating the moisture will be found in Tables XXXIX and XL, pages 279 and 280.

Note.—When a refractometer is available it affords the quickest and most convenient method of determining the water content of most saccharine substances. On sirups and other impure sugar solutions, furthermore, it gives results which are much nearer the actual amount of dry substance than those obtained from specific gravity.

With very dark-colored sirups or solutions, difficulty is sometimes experienced in making readings on account of blurring of the border line due to excessive dispersion. In such cases it is necessary to dilute the solution. Water should not be used for dilution, however, on account of the errors caused by the difference in contraction of volume between sucrose and the accompanying impurities when dissolved in water.

Tischtschenko[1] has suggested the elimination of this error by diluting the sirup with a sucrose solution of about the same refractive index. A weighed amount of the sample is thoroughly mixed with an equal weight of pure sugar sirup whose sugar content is known and the refractive index of the mixture determined. The percentage of solids in the original sirup is then calculated by the formula

$$x = 2P - p$$

where P is the per cent. of solids (from the table) corresponding to the refractive index of the mixed sirups and p is the per cent. of solids in the pure sugar sirup used for dilution.

Polarization.—Use the normal weight of the sample and obtain the direct and invert polarization at 20°C. as directed on page 257. Either 5 cc. of alumina cream or 1–2 cc. of basic lead acetate may be used as a clarifier. Polarization of the inverted solution

[1] *Z. Ver. Deut. Zuckerind.*, **1909**, 103.

TABLE XXXIX.—PER CENT. OF WATER IN SUGAR SOLUTIONS CORRESPONDING TO READINGS OF THE ABBE REFRACTOMETER AT 20°C.[1]

N_D^{20}	Water, per cent.	N_D^{20}	Water, per cent.	N_D^{20}	Water, per cent.	N_D^{20}	Water, per cent.
1.3330	100.0	1.3598	82.5	1.3902	65.0	1.4253	47.5
1.3337	99.5	1.3606	82.0	1.3911	64.5	1.4264	47.0
1.3344	99.0	1.3614	81.5	1.3920	64.0	1.4275	46.5
1.3351	98.5	1.3622	81.0	1.3929	63.5	1.4285	46.0
1.3359	98.0	1.3631	80.5	1.3939	63.0	1.4296	45.5
1.3367	97.5	1.3639	80.0	1.3949	62.5	1.4307	45.0
1.3374	97.0	1.3647	79.5	1.3958	62.0	1.4318	44.5
1.3381	96.5	1.3655	79.0	1.3968	61.5	1.4329	44.0
1.3388	96.0	1.3663	78.5	1.3978	61.0	1.4340	43.5
1.3395	95.5	1.3672	78.0	1.3987	60.5	1.4351	43.0
1.3403	95.0	1.3681	77.5	1.3997	60.0	1.4362	42.5
1.3411	94.5	1.3689	77.0	1.4007	59.5	1.4373	42.0
1.3418	94.0	1.3698	76.5	1.4016	59.0	1.4385	41.5
1.3425	93.5	1.3706	76.0	1.4026	58.5	1.4396	41.0
1.3433	93.0	1.3715	75.5	1.4036	58.0	1.4407	40.5
1.3441	92.5	1.3723	75.0	1.4046	57.5	1.4418	40.0
1.3448	92.0	1.3731	74.5	1.4056	57.0	1.4429	39.5
1.3456	91.5	1.3740	74.0	1.4066	56.5	1.4441	39.0
1.3464	91.0	1.3749	73.5	1.4076	56.0	1.4453	38.5
1.3471	90.5	1.3758	73.0	1.4086	55.5	1.4464	38.0
1.3479	90.0	1.3767	72.5	1.4096	55.0	1.4475	37.5
1.3487	89.5	1.3775	72.0	1.4107	54.5	1.4486	37.0
1 3494	89.0	1.3784	71.5	1.4117	54.0	1.4497	36.5
1.3502	88.5	1.3793	71.0	1.4127	53.5	1.4509	36.0
1.3510	88.0	1.3802	70.5	1.4137	53.0	1.4521	35.5
1.3518	87.5	1.3811	70.0	1.4147	52.5	1.4532	35.0
1.3526	87.0	1.3820	69.5	1.4158	52.0	1.4544	34.5
1.3533	86.5	1.3829	69.0	1.4169	51.5	1.4555[2]	34.0
1.3541	86.0	1.3838	68.5	1.4179	51.0	1.4570	33.5
1.3549	85.5	1.3847	68.0	1.4189	50.5	1.4581	33.0
1.3557	85.0	1.3856	67.5	1.4200	50.0	1.4593	32.5
1.3565	84.5	1.3865	67.0	1.4211	49.5	1.4605	32.0
1.3573	84.0	1.3874	66.5	1.4221	49.0	1.4616	31.5
1.3582	83.5	1.3883	66.0	1.4231	48.5	1.4628	31.0
1.3590	83.0	1.3893	65.5	1.4242	48.0	1.4639	30.5

[1] Schönrock: *Z. Ver. Deut. Zuckerind.*, **1911**, 421.

[2] The values for the refractive index below 34.0 per cent. are taken from Main's table: *Int. Sugar J.*, **9**, 481.

TABLE XXXIX.—PER CENT. OF WATER IN SUGAR SOLUTIONS CORRESPONDING TO READINGS OF THE ABBE REFRACTOMETER AT 20° C.—(*Continued*)

N_D^{20}	Water, per cent.	N_D^{20}	Water, per cent.	N_D^{20}	Water, per cent.	N_D^{20}	Water per cent.
1.4651	30.0	1.4749	26.0	1.4850	22.0	1.4954	18.0
1.4663	29.5	1.4762	25.5	1.4863	21.5	1.4967	17.5
1.4676	29.0	1.4774	25.0	1.4876	21.0	1.4980	17.0
1.4688	28.5	1.4787	24.5	1.4888	20.5	1.4993	16.5
1.4700	28.0	1.4799	24.0	1.4901	20.0	1.5007	16.0
1.4713	27.5	1.4812	23.5	1.4914	19.5	1.5020	15.5
1.4725	27.0	1.4825	23.0	1.4927	19.0	1.5033	15.0
1.4737	26.5	1.4838	22.5	1.4941	18.5	.....	.....

TABLE XL.—STANEK'S CORRECTION TABLE FOR DETERMINING WATER IN SUGAR SOLUTIONS BY THE ABBE REFRACTOMETER WHEN READINGS ARE MADE AT TEMPERATURES OTHER THAN 20°C.

Water, per cent.	95	90	85	80	70	60	50	40	30	25	Water, per cent.
Temperature, °C.	To be added to the per cent. of water.										Temperature, °C.
15	0.25	0.27	0.31	0.31	0.34	0.35	0.36	0.37	0.36	0.36	15
16	0.21	0.23	0.26	0.27	0.29	0.31	0.31	0.32	0.31	0.29	16
17	0.16	0.18	0.20	0.20	0.22	0.23	0.23	0.23	0.20	0.17	17
18	0.11	0.12	0.14	0.14	0.15	0.16	0.16	0.15	0.12	0.09	18
19	0.06	0.07	0.08	0.08	0.08	0.09	0.09	0.08	0.07	0.05	19
Temperature, °C.	To be subtracted from the per cent. of water.										Temperature, °C.
21	0.06	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	21
22	0.12	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.14	0.14	22
23	0.18	0.20	0.20	0.21	0.21	0.21	0.23	0.21	0.22	0.22	23
24	0.24	0.26	0.26	0.27	0.28	0.28	0.30	0.28	0.29	0.29	24
25	0.30	0.32	0.32	0.34	0.36	0.36	0.38	0.36	0.36	0.37	25
26	0.36	0.39	0.39	0.41	0.43	0.43	0.46	0.44	0.43	0.44	26
27	0.43	0.46	0.46	0.48	0.50	0.51	0.55	0.62	0.50	0.51	27
28	0.50	0.53	0.53	0.55	0.58	0.59	0.63	0.70	0.57	0.59	28
29	0.57	0.60	0.61	0.62	0.66	0.67	0.71	0.78	0.65	0.67	29
30	0.64	0.67	0.70	0.71	0.74	0.75	0.80	0.86	0.73	0.75	30
Water, per cent.	95	90	85	80	70	60	50	40	30	25	Water, per cent.

at 87°C. will usually not be necessary unless the direct reading is decidedly higher than the percentage of sucrose, indicating the presence of commercial glucose.

Reducing Sugar.—Dilute 15 cc. of the solution used for polarizing to 50 cc. and determine the reducing sugar by the Munson and Walker method, page 237. Calculate the result as invert sugar, using the column of the table headed "Invert sugar and sucrose, 2 grams total sugar."

Note.—On account of the large proportion of sucrose, determinations of reducing sugar are not accurate unless allowance is made for the reducing action of the Fehling's solution on the sucrose. (See page 239.)

Ash.—Using about 5 grams of the sample, determine the total, water-soluble and water-insoluble ash, and the alkalinity of the water-soluble ash and of the insoluble ash, as described under General Methods, page 16. Express the alkalinity of the ash in each case as the number of cubic centimeters of tenth-normal acid required for the ash of 1 gram of sample.

Note.—Observe carefully the rate of burning and the character of the ash. Adulterated sirups usually burn more slowly and leave particles of unburned carbon. Pure maple ash is gray to green in color and sometimes has a curious network structure like the veining of a leaf.

Lead Number.—When basic lead acetate is added to maple sirup or to a solution of maple sugar, a voluminous precipitate is produced, consisting mainly of the lead salts of malic and other organic acids, of sulphates, chlorides and albuminous matter. This is so constant a function of genuine maple products that the amount and character of this precipitate serves to distinguish them from products containing refined cane sugar, which gives practically no precipitate under similar conditions. The following methods are based on this action with lead acetate.

(*a*) *Winton Method.*[1]—Weigh out 25 grams of the sample in a sugar dish and transfer to a 100-cc. flask with water. With a pipette add 25 cc. of standard lead subacetate solution,[2] shake, fill to the mark, shake, and allow it to stand for at least 3

[1] Winton and Kreider: *J. Am. Chem. Soc.*, **1906**, 1204.

[2] To one volume of the ordinary solution (p. 255) add four volumes of water and filter if not perfectly clear.

hours. Filter through a dry filter, from the clear filtrate pipette 10 cc. into a beaker, add 40 cc. of water and 10 cc. of sulphuric acid (one part concentrated acid to four parts of water). Mix and add 100 cc. of 95 per cent. alcohol. Let stand over night, filter on a Gooch crucible, wash with 95 per cent. alcohol, dry in a water-oven and ignite over a Bunsen burner. Ignite gently at first, then at a low redness for 3 minutes, taking care to avoid the reducing cone of the flame. Cool and weigh.

Blank Determination.—Place 25 cc. of the standard lead solution in a 100-cc. flask, add a few drops of acetic acid and make up to the mark with water. Shake and use 10 cc. for the determination of the lead as directed above.

Calculation.—Subtract the weight of precipitated lead sulphate in the determination from that in the blank, multiply the difference by 0.6829 to obtain the weight of lead, divide by 2.5 and multiply by 100. The result is the "lead number."

Note.—The acid is added in the blank in order to keep the lead from precipitating (as carbonate) when diluted, that is, to imitate the action of pure sugar. Without its use a negative lead number would be possible, especially if the blank solution were filtered. It should be added only to the blank, since if used in the determination itself it would dissolve the lead precipitate.

(*b*). *Ross' Modified Method.*[1]—The lead number obtained by the Winton method on a mixture of maple sirup and cane-sugar sirup is not proportional to the amount of maple sirup present. For example, in an actual experiment a mixture of 50 per cent. of maple sirup having a lead number of 0.90, with 50 per cent. of a cane-sugar sirup of equal specific gravity, showed a lead number of 0.29 instead of the theoretical 0.45. This is due to the solvent action of the sugar solution, partially preventing the formation of the maple lead precipitate, and, according to Ross, can be prevented by the addition of potassium sulphate previous to adding the lead subacetate. The method is as follows: Transfer 25 grams of the sirup to a 100-cc. flask, using about 25 cc. of freshly boiled, distilled water, add 10 cc. of potassium sulphate solution (7 grams per liter); then add 25 cc. of lead subacetate and proceed as in the Winton method. Run a blank in exactly the same way

[1] *Bur. of Chem., Circular* **53.**

using 25 grams of a pure cane sugar sirup (66 per cent. sucrose content) in place of the sirup to be tested. Do not use acetic acid in the blank.

Note.—The lead numbers obtained in this way average about 0.5 higher than by the Winton method (1.8 to 3.0 instead of 1.2 to 2.5) but the results obtained on mixtures are proportional.

(*c*) *Hortvet's Centrifugal Method.*—If a suitable centrifuge is available, a rapid approximation to the purity of a sample may be made by measuring the volume of the lead subacetate precipitate, as suggested by Hortvet.[1]

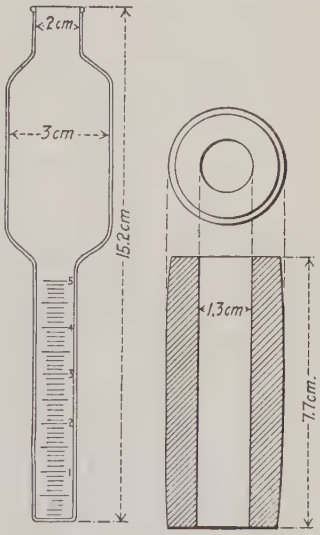


FIG. 52.—HORTVET tube and holder.

Apparatus.—The apparatus required consists of a graduated glass tube and wooden holder, as shown in Fig. 52. These should be balanced in pairs in order to run smoothly in the centrifuge. A machine of the type shown on page 29 is admirably adapted to the determination.

Method.—Place in the tube 5 cc. of sirup or 5 grams of sugar, add 10 cc. of water and stir or shake until dissolved. Add 10 drops of alumina cream and 1.5 cc. of basic lead acetate (see page 255) and shake thoroughly. Allow the tube to stand for an hour, occasionally giving it a twisting motion to assist the settling of the precipitate. Place the tubes and holders in opposite cups of the centrifuge and run for 6 minutes at the proper speed (see Note below). If any material adheres to the upper portion of the tube, loosen it by a small wire provided with a loop at the end. Place the tubes again in the centrifuge and run for 6 minutes longer at the same rate as before. Read the volume of the precipitate, estimating the hundredths of a cubic centimeter. Run blanks at the same time, using water and the reagents used in the determination, and correct for the precipitate given by the re-

[1] *J. Am. Chem. Soc.*, **1904**, 1523.

agents. In the case of a sirup, reduce the results to a 5-gram basis by dividing by the specific gravity. 1.33 will be sufficiently close for ordinary work.

Notes.—The centrifuge used by Hortvet had a radius of 18.5 cm. and was run at a speed of 1600 revolutions per minute. Calling M unity in the formula for centrifugal force, $F = \frac{Mv^2}{r}$, the numerical value for F in this centrifuge would be 519,363, or in round numbers 520,000. In order to obtain comparable results with some other machine of different radius (r), the corresponding velocity (v) in centimeters per second, at the circumference of the wheel, and required number of revolutions per minute (R) should be calculated by the following formulæ:

$$v = \sqrt{520{,}000r}, \qquad R = \frac{60v}{2\pi r}$$

The volume of lead precipitate was found by Hortvet to vary from 0.9 cc. to 1.8 cc. for pure maple sirup and from 1.2 cc. to 4.4 cc. for pure maple sugar. Samples adulterated with cane sugar give much lower results.

The method has the advantage of rapidity but on account of the difficulty in duplicating all conditions exactly, can hardly be considered as accurate as the preceding ones.

In the absence of a centrifuge, the volume of lead precipitate can be measured quite simply as suggested by Sy.[1]

Five cubic centimeters of sirup or 5 grams of sugar are placed in a stoppered measuring cylinder, 10 cc. of water and 2 cc. of basic lead acetate added; mix thoroughly and allow to settle 20 hours; at the end of this time, read the volume of the settled precipitate. For pure maple products this will be over 3 cc., and is usually over 5 cc.

Malic Acid Value.—(*a*) *Calcium Chloride Method.*[2]—Weigh 6.7 grams of the sample in a sugar dish and transfer to a 200-cc. beaker with 15 cc. of water. Add 2 drops of ammonium hydroxide (sp. gr. 0.90); shake, add 1 cc. of a 10 per cent. solution of calcium chloride, then 60 cc. of 95 per cent. alcohol; cover with a

[1] *J. Am. Chem. Soc.*, **1908,** 1430.

[2] Leach and Lythgoe: *J. Am. Chem. Soc.*, **1906,** 380; Hortvet: *J. Am. Chem. Soc.*, **1906,** 1536; *Bur. of Chem., Bull.* **107,** p. 74.

watch-glass and heat on the water-bath for an hour, then turn off the flame and allow the beaker to stand on the bath over night. Filter through good quality filter paper and wash the precipitate with hot 75 per cent. alcohol until the filtrate measures 100 cc.; dry the precipitate and filter and ignite in a platinum dish. Add 10 to 15 cc. of tenth-normal hydrochloric acid to the ignited residue, thoroughly dissolve the lime by heating carefully to just below the boiling point; cool, and titrate the excess of acid with tenth-normal sodium hydroxide, using methyl orange as indicator. Since 1 cc. of tenth-normal acid = 0.0067 gram of malic acid and 6.7 grams of sample were weighed, one-tenth of the number of cubic centimeters of acid neutralized by the ignited residue expresses the malic acid value. Run blanks with each set of determinations, using the same amount of reagents and subtract the result from the malic acid value obtained.

(*b*) *Calcium Acetate Method.*[1]—Weigh 6.7 grams of the sample in a sugar dish. Transfer to a 200-cc. beaker with 5 cc. of water. Add 2 cc. of 10 per cent. calcium acetate solution and shake. Stir in 100 cc. of 95 per cent. alcohol and agitate the solution until the precipitate settles, leaving the supernatant liquid clear. Filter off the precipitate and wash with 75 cc. of 85 per cent. alcohol. Dry the filter paper and ignite in a platinum dish. Add 10 cc. of tenth-normal hydrochloric acid and warm gently until all the lime dissolves. Cool and titrate back with tenth-normal sodium hydroxide, using methyl orange as indicator. One-tenth of the number of cubic centimeters of tenth-normal acid used up is the malic acid number. Run a blank determination and subtract the result obtained from the malic acid number.

Note.—The two methods depend upon the precipitation of malic acid as calcium malate, only slightly soluble in alcohol. This when ignited yields calcium carbonate, which is titrated with the standard acid. The "value" obtained is not the actual percentage of malic acid, since calcium, either as the chloride or acetate, in the presence of alcohol, will precipitate other organic acids. The results are, however, comparable if done under uniform conditions.

The amount of ammonia added greatly influences the results

[1] Cowles: *J. Am. Chem. Soc.*, **1908**, 1285.

and therefore the directions must be followed exactly. The acetate method is shorter and the blanks obtained are more uniform than in the other. The results obtained average about 0.2 higher than by the calcium chloride method.

Preservatives.—Occasionally, maple sirup is found containing an added preservative. The sirup is usually one with an excessive water content and the preservative commonly employed is sodium benzoate, which may be detected and determined as described on page 93.

Interpretation of Analyses

U. S. Standard for Maple Sugar and Sirup.—"*Maple sirup* is sirup made by the evaporation of maple sap or by the solution of maple concrete, and contains not more than 32 per cent. of water and not less than 0.45 per cent. of maple sirup ash.

Maple sugar is the solid product resulting from the evaporation of maple sap, and contains in the water-free substance not less than 0.65 per cent. of maple sugar ash."

Composition of Known Purity Samples.—In Table XLI are collected the results of the earlier analyses of pure maple products

Table XLI.—Compiled Results on Pure Maple Products
(Calculated on Original Substance)

Determination	Maple sugar			Maple sirup		
	Max.	Min.	Av.	Max.	Min.	Av.
Water (per cent.)	11.0	3.05	.....	.....	.....	.....
Direct polarization °V	87.4	72.6	.....	62.2	51.0	.....
Invert sugar (per cent.)	8.37	1.16	.....	9.17	0.34	.....
Lead number	2.48	1.83	2.23	2.03	1.19	1.49
Total ash (per cent.)	1.32	0.64	0.91	1.01	0.46	0.60
Soluble ash (per cent.)	0.67	0.33	0.46	0.63	0.21	0.38
Insoluble ash (per cent.)	0.87	0.20	0.46	0.56	0.14	0.23
Alkalinity of soluble ash	0.95	0.40	0.63	0.68	0.26	0.50
Alkalinity of insoluble ash	1.72	0.55	0.94	0.94	0.31	0.54
Ratio of insoluble to soluble ash	2.2	0.5	1.00	3.2	0.6	1.7
Malic acid value	1.67	0.65	1.01	1.76	0.41	0.78

by Jones,[1] Hortvet,[2] and Winton,[3] and a number of analyses made in the Bureau of Chemistry, in 1904–1905.[4]

In Table XLII are given the maximum, minimum and average results of the analysis of 481 samples of maple sirup of known purity collected from the most important maple-producing districts of the United States and from Canada.[5]

TABLE XLII.—ANALYSES OF MAPLE SIRUP

Determination	Maximum	Minimum	Average
Moisture (per cent.)	48.14	24.85	34.22
Sucrose (per cent.)	70.46	47.20	62.57
Invert sugar (per cent.)	11.01	0.0	1.47
Ash (per cent.)	1.06	0.46	0.66
Direct polarization 20°C.	+ 69.00	+ 42.10	+ 60.64
Invert polarization 20°C.	− 24.97	− 17.00	− 22.34
Invert polarization 87°C.	0.0	0.0	0.0
Calculated to moisture-free basis			
Total ash (per cent.)	1.68	0.68	1.00
Soluble ash (per cent.)	1.23	0.35	0.63
Insoluble ash (per cent.)	1.01	0.23	0.37
Sol. ash ÷ insol. ash	3.86	0.53	1.70
Alkalinity of soluble ash	1.22	0.41	0.75
Alkalinity of insoluble ash	2.08	0.41	0.97
Alk. sol. ash ÷ alk. insol. ash	1.83	0.21	0.77
Lead number	4.41	1.76	2.70
Malic acid value (calcium chloride)	1.60	0.29	0.84
Malic acid value (calcium acetate)	1.82	0.21	1.01

In Table XLIII[6] are given the results of analysis by the same methods of some of the common adulterants.

In the greater number of samples, the two analytical tests which give most readily an idea of the purity of the product are the total ash and the lead number. If the results on a given sample agree with the average values for the pure product given in Tables

[1] *17th and 18th Ann. Rept. Vermont Agr. Expt. Sta.*, 1904–05.
[2] *Loc. cit.*
[3] *Loc. cit.*
[4] *Bur. of Chem., Circ.* **40.**
[5] *Bur. of Chem., Bull.* **134** (1910).
[6] Jones: *Vt. Agr. Expt. Sta. Rept.*, **1905.**

XLI and XLII, it is a fair assumption either that the sample is genuine or that the adulterant is not the usual one of refined cane sugar. Since the addition of refined cane sugar sirup is the most common adulteration, the lowering of the ash and lead number, together with a corresponding decrease in the solubility and alkalinity of the ash, will in the majority of cases be enough to point out the adulterant.

TABLE XLIII.—COMMON ADULTERANTS OF MAPLE SIRUP

Sample	Total ash, per cent.	Soluble ash, per cent.	Insol. ash, per cent.	Alk. of sol. ash, cc.	Alk. of insol. ash, cc.	Ratio of soluble ash : insoluble ash	Malic acid value
Brown sugar:							
Dark	4.33	2.74	1.59	0.76	2.34	1.7:1	.....
Medium	2.80	2.15	0.65	0.15	1.18	3.3:1	.....
Light	0.74	0.68	0.06	0.26	0.15	11.3:1	.....
Raw cane sugar	0.59	0.41	0.18	0.32	0.46	2.3:1	.....
Filtered sirup from same	0.26	0.16	0.10	0.24	0.24	1.6:1	0.35
Beet sugar:							
White	0.33	0.31	0.02	0.40	0.02	15.5:1	.....
Light	0.86	0.78	0.08	0.38	0.28	9.8:1	0.08
Commercial glucose	0.57	0.45	0.12	0.24	0.18	3.8:1	.....

Typical instances of this common form of adulteration, taken from the "Notices of Judgment" published under the Food and Drugs Act, together with the general conclusion upon which prosecution was based, are given in the table on page 289.

The addition of brown sugar instead of granulated sugar is not shown so readily. The dark grades may be detected in some instances by the pronounced flavor which they impart to the product, but the light sugars, which are more commonly used, can be recognized best by the character of their ash. The total ash of the adulterant may not differ greatly from that of the maple product, but the solubility and alkalinity of the ash are quite different, especially when the ratios for these, referred to the soluble and insoluble ash, are calculated.

The lead number and malic acid value of pure maple products are also greatly reduced by the addition of brown sugar. An analysis of the ash itself will give valuable information in doubt-

ful cases. The per cent. of sulphur trioxide and the ratio of calcium oxide to potassium oxide is much higher in brown sugar than in maple sugar.

TABLE XLIV.—ADULTERATED MAPLE SIRUPS

Determination	A	B	C	D	E
Total solids (per cent.)	64.5	68.4	69.3	67.82	66.5
Total ash (per cent.)	0.16	0.59	0.20	0.075	0.08
Soluble ash (per cent.)	.....	0.56	0.12	0.055	0.04
Insoluble ash	.....	0.03	0.08	0.020	0.04
Ratio soluble : insoluble ash	.....	19:1	1.63:1	2.75:1	1:1
Alk. sol. ash (cc. $\frac{N}{10}$ acid)	.....	2.08	1.14	0.025	.....
Alk. insol. ash (cc. $\frac{N}{10}$ acid	.....	0.60	0.16	0.145	.....
Ratio alk. sol. ash: alk. insol. ash	.....	3.5:1	0.875:1	0.17:1	.....
Direct polarization (°V)	+60.5	.....	+59.0	+64.5	.....
Invert polarization (°V at 20°C.)	−20.1	.....	−22.2	−23.6	.....
Invert polarization (°V at 87°C.)	.....	.....	0.0	0.0	.....
Sucrose (per cent.)	61.3	.....	61.20	66.41	.....
Reducing sugar before inversion (per cent.)	.....	.....	8.15	.....	.....
Lead number	0.6	0.52	0.38	0.0	0.11

A.—"Cane-sugar sirup greatly in excess of maple sirup."

B.—"Cane-sugar sirup flavored with some constituent of the maple tree unlike pure sap sirup of live trees."

C.—"Fifty per cent. cane sirup; 50 per cent. maple sirup."

D.—Formula given on can was: Maple sugar, 40 per cent.; cane sugar, 60 per cent. The analysis shows it to contain no appreciable quantity of maple sugar.

E.—"Less than 5 per cent. of maple sirup."

The value of the determinations of solubility and alkalinity of ash in detecting brown sugars is well illustrated in the muscovado sugar mentioned on page 272. These ratios are shown in the following table:

(Calculated to Water-free Basis)

Determination	Muscovado sugar	Maple sugar
Water-soluble ash (per cent.)	1.23	0.50
Water-insoluble ash (per cent.)	0.17	0.64
Ratio $\frac{\text{soluble ash}}{\text{insoluble ash}}$	7.7:1	0.8:1
Alkalinity of soluble ash (cc. $\frac{N}{10}$)	0.11	0.49
Alkalinity of insoluble ash (cc. $\frac{N}{10}$)	0.03	1.47
Ratio $\frac{\text{alk. sol. ash}}{\text{alk. insol. ash}}$	3.7:1	0.33:1

The addition of commercial glucose is readily shown by the abnormally high direct polarization, especially as compared with the calculated percentage of sucrose, and by a decided dextrorotation at 87°C. of the inverted solution. With genuine maple sirup or sugar, the direct polarization usually is slightly *less* than the actual content of sucrose on account of the small amount of invert sugar present; with samples containing commercial glucose the direct reading is decidedly *more* than the sucrose per cent. A slight reading, say of 2° or 3°, to the right at 87°C. on the inverted solution, should not be taken as indicating commercial glucose since this result might easily arise from fermentation of the sample, or from partial destruction of the levulose during inversion and heating.

Honey

Honey, while ordinarily considered as being derived from the nectar of flowers, is more exactly described as "a saccharine product gathered by bees." In addition to the floral nectar, which is the chief source of honey, the bees gather also considerable quantities of various saccharine exudations of leaves and plants which, as will be seen later, materially modify the composition of the final product.

Honey is one of the earliest forms of saccharine food products, and on account of its agreeable flavor has continued in use even after cheaper sources of sugar have been developed.

Chemically, it is a sirup made up mainly of invert sugar with a

varying but relatively small proportion of non-sugars, consisting of mineral matter, proteins, dextrins and usually formic acid. There are usually present also extraneous substances, as pollen from the flowers and wax from the comb.

Sources.—The main source of honey, and the only source of true honey, is the reducing sugar and sucrose in the nectar of flowers, the latter being changed to invert sugar through the action of an inverting enzyme secreted by the bee.

Considerable quantities are derived also from the so-called "honey dew," an exudation which is produced on the surface of the leaves of plants and trees, especially through the agency of the plant aphis. Honey dew differs from floral nectar in the high percentage of ash and dextrins that it contains, being on account of the latter, strongly dextro-rotatory. This, as would be expected, has a decided effect in modifying the physical and chemical properties of the resultant honey.

Composition.—The most extended investigation into the composition of American honeys[1] has been made by Browne[2], from whose work the following figures are taken. In the table are included also data on seventy-two samples of honey imported from Cuba, Haiti and Mexico, which closely resemble the American honeys.[3]

Forms of Adulteration.—The forms of adulteration ordinarily found consist in the presence of excessive amounts of sucrose, either added as such or introduced by feeding it to the bees; in the admixture of commercial glucose in greater or less quantity; and in the substitution in part of artificial invert-sugar sirups. The presence of too much water is, of course, also an adulteration.

Methods of Analysis

Preparation of the Sample.—If the honey is clear and liquid, it is ready for analysis without further treatment. If it has granulated and sugar has separated, place the bottle in a bath of water

[1] Numerous analyses of European honey, which differs slightly from the American product, may be found in König: *Chemie der menschlichen Nahrungs- und Genussmittel.*

[2] *Bur. of Chem., Bull.* **110.**

[3] Bryan: *Bur. of Chem., Bull.* **154,** p. 9.

TABLE XLV.—ANALYSES OF AMERICAN AND IMPORTED HONEYS

Determination	Levo-rotatory honeys (92)			Dextro-rotatory honeys (7)			Imported honeys (72)		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
Direct polarization, 20°C. (immediate), °V.	−21.9	+ 3.7	−11.24	+24.9	+ 6.7	+14.77	−22.90	− 6.05	−13.34
Direct polarization, 20°C. (constant), °V	−24.8	− 0.3	−14.73	+17.75	+ 3.6	+ 9.43	−24.15	− 8.50	−14.52
Direct polarization, 87°C., °V	+23.7	+ 0.5	+10.15	+35.8	+28.5	+32.20	+17.00	+ 3.20	+10.31
Invert polarization, 20°C., °V.	−29.26	− 1.32	−19.16	+14.96	− 2.53	+ 5.47	−26.07	− 8.86	−16.22
Invert polarization, 87°C., °V.	+23.21	− 0.66	+ 7.91	+34.98	+20.90	+27.56	+15.40	+ 2.86	+ 9.08
"Difference", °V	33.55	23.32	27.07	23.43	20.02	22.09	28.93	22.77	25.30
Water (per cent.)	26.88	12.42	17.70	17.80	13.56	16.09	27.00	16.05	21.26
Invert sugar (per cent.)	83.36	62.23	74.98	71.69	64.84	66.96	77.56	68.09	72.38
Sucrose (per cent.)	10.01	0.00	1.90	5.28	0.61	3.01	3.98	0.00	0.80
Ash (per cent.)	0.90	0.03	0.18	1.29	0.29	0.81	0.58	0.06	0.21
Dextrin (per cent.)	7.58	0.04	1.51	12.95	6.02	9.70	3.96	0.26	1.24
Undetermined (per cent.)	7.45	0.04	3.73	4.95	1.57	3.43	8.07	0.66	4.11
Free acid as formic (per cent.)	0.25	0.04	0.08	0.19	0.05	0.12	0.43	0.00	0.15
Reducing sugars as dextrose (per cent.)	79.86	59.61	71.08	68.68	62.12	64.15	.....	.....	.....

at 45° to 50°C. until it is again entirely liquid. Remove any appreciable amount of wax or other foreign matter by straining through a cloth or fine sieve.

Note.—The granulation of honey, which is of common occurrence, is due to the separation in a crystalline mass of the dextrose, leaving in solution an excess of the less readily crystallizable levulose.

Water.—Because of the decomposition of levulose at temperatures above 70°C., the determination of water should not be made by direct drying, unless a suitable vacuum oven, in which the drying may be made at 70° under reduced pressure, is available. (See page 16.) Either method (*b*) or (*c*) as given on pages 273 and 278 for maple sirup may be employed, although Bryan[1] has found that the results obtained with the refractometer are somewhat high in the case of honey.

Ash.—Use 5 grams of the sample and determine the ash as directed under General Methods, page 16.

Polarization.—(*a*) *Direct.*—Use the normal weight of sample and proceed as directed on page 254 using, however, 5 cc. of alumina cream instead of lead subacetate as a clarifier. To obtain the constant rotation, add two or three drops of ammonia before making up to the mark. (See Mutarotation, page 248.) Polarize the solution at 20°C. Save a portion of this solution for the determination of reducing sugars.

(*b*) *Invert.*—The inversion, if done with care, may be made by the Herzfeld method as described on page 257, noting that the addition of potassium oxalate is in this case not necessary. Owing, however, to the large proportion of levulose present, it is much better to follow the method of inverting by standing at room temperature over night, as given on page 258. After polarizing the inverted solution at 20°C., measure out 50 cc. into a 100-cc. flask, nearly neutralize the acid and polarize at 87°C., as directed on page 260. (Save a portion of the inverted and neutralized solution for the determination of the reducing sugar after inversion.)

Reducing Sugars.—Pipette 10 cc. of the solution used for direct polarization into a 250-cc. graduated flask, make up to the

[1] *J. Am. Chem. Soc.*, **1908**, 1443.

mark and determine the reducing sugars in 25 cc. by the Munson and Walker method (page 237). Calculate the result as invert sugar.

To determine the *reducing sugars after inversion,* pipette 25 cc. of the solution prepared for polarization at 87° into a 250-cc. flask, dilute to volume and determine the reducing sugars in 25 cc. as in the preceding paragraph.

Sucrose.—This should be calculated from the difference in percentage of reducing sugars before and after inversion (see page 245) rather than from the polarization. This is because of the error due to the change in specific rotation of levulose in neutral and in acid solution, which may amount to 1 per cent. or more, an amount which might be sufficient to condemn unjustly a honey for excessive sucrose content.

Qualitative Tests for Adulterants.—Beckmann Test for Commercial Glucose.[1]—To 5 cc. of honey add an equal volume of water and then add a solution of iodine in potassium iodide, a few drops at a time, with frequent shaking, noting any change in the color of the solution to red or violet. Compare the color with a similar test made on pure honey, using the same amount of iodine solution.

Notes.—The test depends upon the presence in commercial glucose of erythro-dextrin, which is one of the intermediate products formed in the acid conversion of starch and gives a red color with iodine. It is limited somewhat by the fact that high converted glucose, in which the conversion has proceeded nearly to the final stages, does not always give a reaction with iodine.

The test may be made more sensitive by precipitating the dextrins of the honey with strong alcohol, dissolving the precipitate in a few cubic centimeters of water and testing with iodine as before.

Anilin-acetate Test.[2]—The reagent used, which should be freshly prepared each time, is made by shaking 5 cc. of pure anilin with 5 cc. of water and adding enough glacial acetic acid (2 cc.) to just clear the emulsion.

To make the test place 5 cc. of a concentrated solution of the honey (1 part of honey to 1 of water) in a test-tube and pour 1 to

[1] *Z. anal. Chem.*, **1896**, 267.

[2] Browne: *Bur. of Chem., Bull.* **110**, p. 68.

2 cc. of the anilin reagent carefully down the side of the tube so as to form a layer above the honey solution. Gently agitate the tube and note whether a red ring forms beneath the anilin solution, the color being in some cases gradually imparted to the whole lower layer.

Fiehe's Test.[1]—Place 5 cc. of honey in a test-tube, add 5 cc. of water and mix thoroughly. Add 5 cc. of ether, shake the tube vigorously and allow to stand until the ether layer is perfectly clear. Transfer 2 cc. of the clear ether solution to a small test-tube and add a large sized drop of resorcin solution (1 gram in 100 cc. of hydrochloric acid). Shake and note the appearance of a red color.

Notes.—Both this reaction and the preceding one are tests for *oxymethylfurfural*, a decomposition product of levulose, and are taken as indicating the presence of artificial invert sugar. This is made on a commercial scale by heating sucrose with a small amount of tartaric or citric acid to about 115°C., under which conditions traces of oxymethylfurfural are formed.

Honey which has been boiled or heated for some time will give a similar reaction, but this is seldom done with the commercial product because it impairs the appearance and flavor.

Microscopical Examination.—Dilute a small portion of the honey with three or four times its volume of water and centrifuge. Place the sediment on a clean microscope slide, cover with a cover glass and examine with a magnification of about 250 diameters. Detailed descriptions and illustrations of the most commonly occurring pollens, together with an analytical key for their identification, will be found in Bulletin 110 of the Bureau of Chemistry.

Note.—The identification of the pollen is of considerable value in determining the correctness of labeling of a sample for which a particular floral source is claimed.

Interpretation of Results

The Federal standards define honey as "the nectar and saccharine exudations of plants gathered, modified, and stored in the comb by honey bees (*Apis mellifica* and *A. dorsata*); it is

[1] Fiehe: *Z. Nahr. Genussm.*, **1908**, 75.

levo-rotatory, contains not more than twenty-five (25) per cent. of water, not more than twenty-five hundredths (0.25) per cent. of ash, and not more than eight (8) per cent. of sucrose."

Cane Sugar.—The average sucrose content of the ninety-nine honeys analyzed by Browne (*loc. cit.*) was 1.98 per cent., and of the whole number only two exceeded the Federal standard of 8 per cent., so that this may be considered a liberal allowance and amounts exceeding this should be regarded as added sugar. The addition of cane sugar in such small quantities that the total amount present does not exceed 8 per cent. obviously cannot be shown by analysis. The same remarks apply to sucrose added by feeding cane sugar to the bees. The latter practice, however, is not common, being unprofitable commercially.

Commercial Glucose.—This may be added to honey in considerable quantities simply as a cheaper substitute, or used in lesser amounts to modify and improve the color and taste of dark-colored and strongly flavored grades of natural honey. The addition of a comparatively small proportion of glucose prevents the granulation of the honey. For whatever purpose added, the presence and amount must under the Federal regulations be declared on the label.

For the detection of commercial glucose in honey, especially if present in small amounts, the method of polarizing the invert solution at 87°C., as recommended for maple sirup and jams, is not suitable. This is due to the fact that genuine honey is nearly always dextro-rotatory under these conditions, the values given in the table on page 292 ranging from +23.21 to a minimum of −0.66 for the levo-rotatory honeys alone. For this reason the invert polarization at 87°, unless distinctly more dextro-rotatory than the maximum value, should be interpreted with extreme caution, and chief reliance placed on the erythro-dextrin test on the honey or on the precipitated dextrins.

For the same reason the method of calculating the amount of commercial glucose from the invert polarization at 87°, as described on page 262 can be taken only as an approximation in the case of honey.

Another method of calculation, which gives somewhat better results, is based upon the variation of the invert polarization of

the sample from an assumed value for pure honey. For example, taking $-17.5°$ as the average invert polarization (at 20°C.) of the honeys in Table XLV and $+175°$ as the average polarization of commercial glucose (see page 262), let

x = per cent. of honey in the sample
y = per cent. of commercial glucose in the sample
P = invert polarization for normal weight of sample

Then

$$x + y = 100$$
$$-0.175x + 1.75y = P$$

Whence

$$y = \frac{P + 17.5}{1.93}$$

An objection to this method, however, lies in the very considerable variation in the invert polarization of genuine honey itself, the extremes of Table XLV showing values of -29.26 to $+14.96$.

A still better method is the one suggested by Browne (*loc. cit.*) based on the difference in the invert polarizations at 20° and 87°. Browne has found that while the invert polarizations at 20° and 87° are subject to wide variations, the difference between the two polarizations is much more nearly constant. Since this difference in polarization is due entirely to the decreased rotation of levulose with increased temperature, in other words, is dependent entirely upon the percentage of invert sugar present, the addition of commercial glucose will lower the polarization difference by an amount proportional to the quantity of commercial glucose added, but irrespective of its specific rotation. On account of the variations in water content and non-sugar solids of pure honey, it is best in applying this method of calculation to reduce the polarization difference to a uniform basis of 77 per cent. reducing sugars, which is the average amount of invert sugar after inversion in pure honey. Taking the average value found by Browne for the polarization difference in pure honey as 26.7, the expression would become

TABLE XLVI.—POLARIZATION OF HONEY AND GLUCOSE MIXTURES

Sample	Direct polarization, 20°C., °V	Invert polarization, 20°C., °V	Invert polarization, 87°C., °V	Polarization difference (87°–20°), °V	Invert sugar after inversion, per cent.	Pol.-diff. corrected to 77 per cent. invert sugar, °V	Glucose from invert polarization at 87°C., per cent.	Glucose from invert polarization at 20°C., per cent.	Glucose from polarization difference, per cent.
Alfalfa honey	−19.5	−22.66	+ 3.52	26.18	77.84	25.90	2.16	0.00	3.00
Alfalfa honey + 20 per cent. glucose	+19.4	+16.88	+35.82	18.94	70.01	20.83	21.97	17.82	21.98
Hop-vine honey	−12.6	−16.83	+ 9.68	26.51	75.83	26.92	5.94	0.35	0.00
Hop-vine honey + 20 per cent. glucose	+24.9	+ 2.54	+40.74	19.20	68.14	21.70	25.00	20.28	18.72
Basswood honey	− 0.3	− 1.32	+23.21	24.53	70.60	26.75	14.24	8.40	0.00
Basswood honey + 20 per cent. glucose	+ 3.48	+33.94	+51.57	17.63	63.97	21.22	31.64	26.72	20.52
White oak honey	+11.0	+ 5.17	+28.60	23.42	70.44	25.61	17.56	11.23	4.08
White oak honey + 20 per cent. glucose	+43.8	+39.14	+55.88	16.74	63.84	20.20	34.28	29.35	24.35

$$H = \frac{100(P^1 - P)}{26.7} \times \frac{77}{I}$$

where

H = per cent. of pure honey in the sample
P^1 = invert polarization at 87°C.
P = invert polarization at 20°C.

and

I = per cent. of invert sugar after inversion

whence

$100 - H$ = per cent. of commercial glucose

It will be seen by examination of the table on page 298 in which Browne has compared these three methods of calculation on known mixtures of commercial glucose with different honeys, both levo-rotatory and dextro-rotatory, that with the latter especially, the calculation from the polarization difference gives results on the whole closer to the truth. It is apparent, further, that none of the methods are by any means exact, and that positive results on a dextro-rotatory honey should not condemn it unless confirmed by the qualitative tests. If the sample contains added commercial glucose there will in nearly every case be a noticeable depression of the "polarization difference" (corrected to 77 per cent. of invert sugar) and a positive reaction of the honey and of its precipitated dextrins toward iodine.

The following table shows the analytical results given by a honey containing a large (50 per cent.) and a small (5 per cent.) proportion of commercial glucose.

TABLE XLVII.—ANALYSES OF HONEY ADULTERATD WITH COMMERCIAL GLUCOSE

	A Honey + 50 per cent. commercial glucose	B Honey + 5 per cent. commercial glucose
Direct polarization 20°C.	+67.0	−11.5
Invert polarization 20°C.	+65.67	−14.31
Invert polarization 87°C.	+73.81	+11.66
Polarization difference	8.14	25.96
Invert sugar before inversion (per cent.)	53.67	75.74
Invert sugar after inversion (per cent.)	54.50	77.80
Water (per cent.)	20.52	19.76
Ash (per cent.)	0.49	0.22

Invert-sugar Sirup.—The addition of invert-sugar sirup constitutes a form of adulteration which is quite difficult of detection because the analytical constants of the adulterant so closely resemble those of honey itself. The product obtained, for instance, by heating a sugar sirup with 0.1 per cent. of citric or tartaric acid is almost identical in chemical composition with honey. The most satisfactory tests for the presence of such a sirup in honey depend upon the formation of traces of some characteristic product by the heating with acid. The best known of these are Fiehe's test and the reaction with anilin-acetate described on page 294. If both of these tests give a positive result when carefully compared with similar tests on pure honey, it may safely be concluded that artificial invert sugar is present.

Selected References

Browne.—Chemical Analysis and Composition of American Honeys. U. S. Dept. of Agr., Bur. of Chem., Bull. 110.

Browne.—Handbook of Sugar Analysis.

Bryan.—Chemical Literature on Honey from 1892 to 1911. U. S. Dept. of Agr., Bur. of Chem., Bulls. 110 and 154.

Bryan.—Maple-sap Sirup. U. S. Dept. of Agr., Bur. of Chem., Bull. 134. (Bibliography of maple products.)

Landolt.—(Trans. by Long) Optical Rotation of Organic Substances.

von Lippman.—Chemie der Zuckerarten.

Rolfe.—The Polariscope in the Chemical Laboratory.

Tollens.—Handbuch der Kohlenhydrate.

Wiechmann.—Sugar Analysis.

Wiley.—Agricultural Analysis, Vol. III.

CHAPTER VII

COCOA AND CHOCOLATE

Source.—Cocoa, chocolate and their preparations are derived from the seeds of the *Theobroma Cacao*, a tree which is cultivated in many semi-tropical countries, especially in South America, the West Indies, Java and Africa. The seeds or "cocoa beans," as they are commonly called, appear in commerce under various names, derived mostly from the district in which they are grown or the port from which they are shipped. Among the more important varieties are: Ariba (Ecuador), Bahia (Brazil), Caracas (Venezuela), Ceylon, Java, Surinam (Dutch Guiana), Trinidad (W. I.), and St. Thomé (Africa).

The seeds are borne in pods about 6 to 10 in. long and shaped something like an overgrown cucumber. These contain from 25 to 75 of the almond-shaped seeds imbedded in a mass of cellular pulp. The pods are cut open and the beans, separated from the pulp, are subjected to a sort of fermentation or "sweating" process for several days, during which the temperature rises somewhat. The results of this treatment are the development of the flavor to a considerable extent, the change of color to the familiar rich chocolate tint and the hardening of the shell. After drying, either in the sun or by artificial heat, the beans are ready for shipment.

Manufacture.—In the manufacture of chocolate the cleaned and sorted beans are roasted at a temperature of 140°C. to 300°C., thereby developing the aroma of cocoa through changes brought about in the essential oils. Another important result of the roasting is the drying of the shell, facilitating its removal, which is done by a thorough winnowing of the crushed beans. The roasting process is of great importance since it is the chief factor in producing the fine flavor of the chocolate.

The crushed beans, freed from shells, are sometimes sold without further treatment as "cocoa nibs" or "cracked cocoa," but by far the greater part are ground between stones in steam-heated mills. Only a slight warming of the stones by the steam

coils at the start is required, the heat of grinding and the high fat content being sufficient to keep the material liquid. The resultant thin paste is run into molds and, after hardening, constitutes the ordinary "plain," "bitter" or unsweetened chocolate.

Sweet chocolate is made by mixing powdered sugar and flavoring, usually vanilla, with the warm chocolate paste in a special mill or "melangeur." The mixture is hardened in molds as in the case of the plain chocolate. Cocoa, called also "breakfast cocoa" or "cocoa powder," is prepared by expressing a portion of the fat or "cocoa butter" from the warm cocoa mass in a hydraulic press. The residue left in the press is then crushed, ground and sifted until it becomes a very fine powder. Alkalies or other chemicals are sometimes added during the process, producing the so-called "Dutch process cocoa." (See page 305.)

Milk chocolate is a somewhat more recent cocoa preparation and is made by adding to the warm chocolate mass either specially prepared condensed milk or dry milk powder.

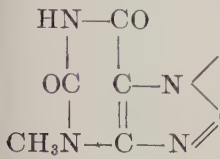
The manufacturing process, although largely mechanical, requires skilled supervision to bring about the best results, and the proper blending of the different varieties of cocoa beans in order to yield the special flavor desired can be learned only by long experience. The differing degrees of softness of the chocolate, as required for the various uses to which it is put by the confectioner, are attained by mixing in the necessary proportion of the "butter" expressed in the manufacture of cocoa.

Composition.—The most important constituents of the cocoa bean are the fat, which makes up about half the weight of the bean, protein, starch, theobromine and caffeine and a peculiar pigment known as "cocoa red." The fat has considerable commercial use in addition to its use by the cocoa manufacturer in blending or softening his products, being quite largely employed in pharmacy. It is not usually regarded as an edible fat when in a pure condition on account of its indigestibility, although when in the form of chocolate it is stated to be nearly as digestible as milk fat.[1]

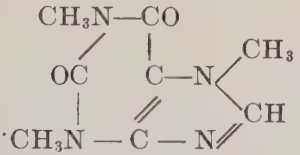
The stimulating effect of cocoa as a beverage is due mainly to the alkaloids, theobromine and caffeine, from 0.9 to 3.0 per cent.

[1] Zipperer: The Manufacture of Chocolate, p. 44.

of the former and 0.05 to 0.35 per cent. of the latter being present. The theobromine is found in the bean in two forms, partly in combination with dextrose and the cocoa red as a glucoside, and partly as free theobromine, having been set free from the glucoside during the fermentation process. Chemically it is closely related to caffeine, of which it is a lower homologue. Theobromine is 3,7–dimethyl-xanthine,



, while caffeine is 1,3,7–trimethyl-xanthine,



If to a minute quantity of either caffeine or theobromine in a porcelain dish is added a few drops of strong hydrochloric acid and a small crystal of potassium chlorate, and the mixture is evaporated to dryness on the water-bath, a reddish yellow or pink spot is left. On moistening this, when cold, with a few drops of ammonia, a beautiful purple color is produced, the so-called "murexide" test.

The cocoa bean also contains 2 to 5 per cent. of a peculiar and interesting pigment, the "cocoa red," which is formed during the drying of the white beans by the action of enzymes on the glucosides. It is not only a prominent factor in determining the peculiar odor and taste of the cocoa bean, but is also of great importance to the manufacturer, since it determines very largely the color of the finished product. The general composition of the cocoa bean is summarized in the following analysis by Zipperer:[1]

	Per cent.		Per cent.
Moisture	6.3– 8.5	Cocoa red	2.5– 5.0
Fat	46.9–52.1	Ash	2.9– 4.8
Albuminoids	11.6–21.1	Astringent matters	7.2– 8.6
Cellulose	3.3– 6.6	Cane sugar	
Alkaloids	0.3– 0.5	Starch	8.7–12.6

[1] *Loc. cit.*

A somewhat more detailed analysis, by modern methods, and showing the effect of roasting on the bean, is given in Table XLVIII.[1]

TABLE XLVIII.—ANALYSES OF RAW AND ROASTED COCOA BEANS AND SHELLS

	Raw nibs, per cent.	Roasted nibs, per cent.	Raw shells, per cent.	Roasted shells, per cent.
Water	5.13	3.71	8.69	6.01
Total ash	3.05	3.14	11.40	12.04
Ash soluble in water	1.39	1.45	3.63	4.24
Ash insoluble in acid	0.02	0.00	4.59	4.55
Alkalinity of ash (cc. $\frac{N}{10}$ acid)	2.35	2.50	5.32	5.35
Theobromine	1.03	1.02	0.33	0.39
Caffeine	0.42	0.41	0.20	0.21
Other nitrogenous substances	11.38	11.56	12.50	12.69
Crude fiber	1.90	2.71	13.41	15.55
Starch by acid hydrolysis	10.14	10.00	11.35	10.37
Starch by diastase	6.93	7.41	4.59	4.37
Other nitrogen-free substances	18.71	18.39	44.61	45.99
Fat	51.45	51.65	4.27	2.68
Total nitrogen	2.26	2.29	2.16	2.24
Direct polarization (°V)	0.0	0.0	+4.0	+5.0
Invert polarization, 20°C. (°V)	0.0	0.0	+4.0	+4.8
Melting point of fat, (°C.)	33.0	32.7	.....	.....
Refractive index, 40°C.	1.4576	1.4576	.....	.....
Iodine number	36.33	35.61	.....	.....

A discussion of the differences in composition between the nibs and the shells, and of the composition in relation to the conclusions to be drawn from the analytical results, will be found under the Interpretation of Results.

Forms of Adulteration.—The most common adulterations of *plain or bitter chocolate* are the addition of foreign starches, as wheat or arrowroot, or the inclusion, either by accident or intentionally, of a portion of the shells. Occasionally a portion of the fat is found to have been removed, or when the proper amount of fat is found, its constants show that a part of the cocoa butter has been replaced by cheaper substitutes of which there are several on the market, based on the use of cocoanut oil. In Germany, so-called "fat-sparers" consisting of gelatin,

[1] Winton, Silverman and Bailey: *Rept. Conn. Exp. Sta.*, **1902**, p. 268.

dextrin or gum tragacanth, have been used to increase the mucilaginous character of the chocolate when dissolved, or its smoothness when tasted, and thus conceal the deficiency of fat. Very rarely samples have been found in which some mineral adulterant such as red ochre, has been used to add weight or improve the color, coal-tar dyes being also employed for the latter purpose. It has until recently been a common practice, especially in the summer, to coat the cakes of chocolate with some varnish to prevent their softening in warm weather. Gum benzoin, wax and even shellac have been used for this purpose, especially with cheap chocolate candies, but are less commonly employed at present, owing to the activity of the Federal authorities.

In the case of *sweet chocolate* or "eating chocolate," which is usually only the plain chocolate with the addition of sugar and flavoring, another form of adulteration is the addition of sugar in excessive amounts. The amount should not ordinarily be above 60 per cent., although percentages as high as even 90 per cent. have been found.

With *cocoa*, the opportunity and the temptation to use various powdered adulterants is even greater. Such adulterants as corn, sago and arrowroot starch, cocoa shells, wood fibers, and ground acorns are readily detected by the microscope. There is, of course, no objection to the sale of cocoa containing foreign starches or sugar provided it is properly labeled. The same cannot be said of cocoa shells, however, which are largely indigestible and are added solely for fraudulent purposes.

The "soluble cocoas" on the market are prepared by heating the beans with steam under pressure, or by treating the roasted beans or ground cocoa with some form of alkali, the object being to saponify the fat to a certain extent and render it less likely to separate in the cup. By the action of the alkali, also, the vegetable tissues are partly disintegrated and the material will remain suspended in water or milk to a greater degree. The cocoa is darkened in color and given the appearance of greater strength. It should, in fairness, be remarked that the process is considered by many to improve the flavor. In the so-called "Dutch process" sodium, potassium or magnesium carbonate is employed, while in the German method ammonia

or ammonium carbonate is the alkali commonly used. Actual determinations of the solubility of a number of the brands for which special claims are made in this regard, show that the use of the word "soluble" on the label is based more on fiction than on fact. (See page 329.)

Sweetened cocoas, as in the case of sweet chocolate, should be examined for the presence of excessive amounts of sugar, the quantity which can be added legally being limited to 60 per cent. Sometimes cocoa, either sweetened or unsweetened, is found erroneously labeled "powdered chocolate," a term which is absurd upon the face of it since chocolate, which word without qualification means legally plain chocolate or "cocoa mass," can be prepared only in the form of paste and moulded into cakes. Saccharin is occasionally, although not commonly, used in place of sugar in these preparations.

Milk chocolate should be made by adding to the chocolate either condensed milk or milk powder which has been prepared from standard milk containing the full proportion of fat. Such preparations, however, are occasionally made from skimmed milk, or mixtures of starch and powdered cocoa shells are added to cheapen the product.

Methods of Analysis

Preparation of Sample.—Cocoa, in the state of a fine powder, requires no preliminary treatment. Chocolate or chocolate preparations in cake form should be carefully shaved with a knife, or may be thoroughly chilled on the ice and grated with a nutmeg grater, also chilled. In either case endeavor to take a representative portion of the cake and handle it with the hands as little as possible.

Moisture.—Two grams of the sample are weighed out and dried in the water-oven to constant weight, either directly or after mixing with about 10 grams of ignited sand.

Ash Data.—Use 2 grams of sample and determine the total ash, ash insoluble in water and ash insoluble in acid exactly as described under General Methods, page 16. Titrate the filtrate from the ash insoluble in water with tenth-normal hydrochloric acid, as stated on page 19, and report the alkalinity of the soluble

ash as cubic centimeters of acid required for the ash of 1 gram of sample. For comparison with older reported analyses, it may be advisable also to determine the alkalinity of the total ash in the same manner, reporting the results in cubic centimeters of tenth-normal acid as before.

Fat.—Extract 2 grams of the dried sample in a continuous extraction apparatus with anhydrous ethyl ether until no more fat is removed. Grind the residue and repeat the extraction. Evaporate the ether, dry the combined extracts at 100°C. and weigh.

Notes.—The above method is that recommended provisionally by the Association of Official Agricultural Chemists. The material to be extracted should be wrapped in double filter papers before being placed in the extracting apparatus, since the particles of some chocolates are extremely small and considerable will pass through an ordinary filter. Low results are always obtained, on the other hand, if the chocolate is not in this extremely fine state of division.

With most cocoa products the fat can be determined with sufficient accuracy for ordinary purposes without the re-extraction. Experiments in this laboratory[1] have shown that in the case of cocoa, extraction for 6 hours gave 99.7 per cent. and for 8 hours 99.92 per cent. as much ether extract as was obtained by 16 hours extraction including a re-grinding of the residue. In any case an extraction for 5 hours, followed by grinding of the residue with an equal quantity of fine sand and re-extraction for 2 hours, ought to prove sufficient.

Farnsteiner[2] finds that the fat is completely extracted by ether after 4 hours, and that the material extracted by continuing the process is not fat but mainly theobromine. For this reason, petroleum ether, in which theobromine is insoluble, is preferred by some analysts.

For powdered cocoa products the rapid method described on page 25 will be found especially suitable.

Crude Fiber.—Either use the residue from the determination of fat or treat a 2-gram portion of the original sample directly with ether and proceed as directed on page 269. Since cocoa,

[1] Unpublished results by F. H. Pendleton.

[2] *Z. Nahr. Genussm.*, **1908**, 627.

especially, is liable to clog the Gooch crucible in the final filtration and filter very slowly, decant carefully and keep the insoluble residue from the crucible as long as possible.

Note.—Powdered cocoa is ordinarily fine enough to be used directly, but if the product seems at all granular it is best to cover the weighed sample with ether in a porcelain mortar or metal "sugar dish" and grind it thoroughly under ether as under starch, below. Transfer carefully to a filter, wash several times with ether, dry the residue on the filter and wash it into the 500 cc. flask with a stream of boiling 1.25 per cent. sulphuric acid from a wash bottle. Then proceed as in the regular method. If the sample is not finely ground, the results may be much too high.

Starch.—(*a*) *By Acid Hydrolysis.*—Weigh 4 grams of the sample into a small porcelain mortar (a sugar dish as described on page 255 will be found much more convenient), add 25 cc. of ether and grind with a pestle. After the coarser material has settled out, decant off the ether with the fine suspended matter on an 11-cm. filter. Repeat this treatment until no more coarse material remains. After the ether has evaporated, transfer the fat-free residue from the filter to the mortar by means of a jet of cold water, and rub to an even paste. Filter the liquid on the paper previously employed. Repeat the process of transferring from the filter to the mortar, grinding, and filtering, until all sugar is removed. In the case of sweetened cocoa products, at least 500 cc. of wash water should be used.

Transfer the residue to a 250-cc. flask by means of 200 cc. of hot water, and hydrolyze the starch by Sacchsse's method as detailed on page 263. Cool the acid solution, nearly neutralize with strong sodium hydroxide, add 5 cc. of basic lead acetate solution, make up to 250 cc., mix thoroughly and filter through a dry filter. Remove the lead from the filtrate by adding powdered potassium oxalate in slight excess, filter through a dry filter and determine dextrose in 100 cc. of the filtrate by the Munson and Walker method, page 237.

Notes.—The above method is the one used by Winton and his associates in their study of authentic cocoa beans[1] and has been adopted by the Association of Official Agricultural Chemists as a provisional method. The method will undoubtedly give

[1] *Ann. Rept. Conn. Agr. Expt. Sta.*, **1902.**

accurate results if used with care, but is slow and tedious in the extreme. With sweetened samples it often takes two days to wash with 500 cc. of water, and in warm weather there is danger of the sample molding during that time. The grinding under ether and transferring back and forth from the filter to the mortar must be done with great care to avoid loss.

If a suitable centrifuge is available the following method[1] will be found a great saving of time:

Transfer 4 grams (or 8 grams of sweetened cocoa products) to an 8-ounce nursing bottle or other receptacle which can be used in the centrifuge. Add 50 cc. of gasoline or petroleum ether and shake until the material is completely disintegrated: centrifuge until clear and either decant the gasoline or draw it off by suction. Repeat the process and finally expel the remaining solvent by standing the bottles on the steam-bath for a short time. After thus removing the fat, wash the residue, in the case of unsweetened cocoa, into a 250-cc. graduated flask with 200 cc. of water and proceed as on page 263, except that 10 cc. of basic lead acetate should be added after neutralizing before completing to volume.

With sweetened cocoa products, add to the fat-free residue 5 cc. of alumina cream (page 255) and 100 cc. of water, shake until thoroughly mixed, and centrifuge until the supernatant liquid is clear. Draw this off and repeat the shaking and centrifuging with water twice. Transfer the residue to a 250-cc. graduated flask with 200 cc. of water and proceed as above.

Note.—The repeated treatment with water is in order to remove sugar, the alumina cream being added to assist in the sedimentation of the fine particles of cocoa.

(*b*) *By Diastase.*—Treat 4 grams of the sample with ether and water as on page 308, taking especial care that the material is ground to a fine powder with ether. Carefully wash the wet residue from the paper into a beaker with 100 cc. of water and carry out the hydrolysis as described on page 308, except that as cocoa starch is possibly somewhat more resistant to boiling water than other starches, the boiling with water should be for half an hour and the digestion with malt extract should be continued for 2 hours.

Note.—The percentage of starch, as determined by acid hydrolysis, is distinctly higher than the result of the diastase method,

[1] Dubois: *Bur. of Chem., Bull.* **132,** p. 136; *Bull.* **162,** p. 132.

as would be expected from the considerable amount of non-starchy carbohydrates present. With cocoa nibs the average values for the actual starch content, as shown by the diastase method, are about 3 per cent. lower than by acid hydrolysis. (See Table XLVIII, page 304.) For cocoa shells, the difference is even greater, being about 7.5 per cent.

Sugars.—For the determination of sucrose in sweet chocolate or cocoa, and of sucrose and lactose in milk chocolate, the following general method[1] will be found satisfactory:

Place the normal weight (see page 253) of the finely divided material in an 8-ounce nursing bottle, add about 100 cc. of petroleum ether and shake for 5 minutes to disintegrate the chocolate. Whirl the bottle in a centrifuge until the petroleum ether is clear. Decant carefully, or draw off by suction, and repeat the treatment with petroleum ether. Place the bottle containing the defatted residue in the steam-bath or in a warm place until the petroleum ether is practically entirely expelled. Add exactly 100 cc. of water and shake until the chocolate is loosened from the sides and bottom of the bottle and then for 3 minutes longer. Add from a pipette 10 cc. of basic lead acetate solution, mix thoroughly and filter through a dry filter. Add powdered potassium oxalate to remove the excess of lead, filter again through a dry filter, and polarize in a 200-mm. tube at 20°C. Invert 50 cc. of the filtrate, preferably using the method of inversion in the cold (page 258) and a flask graduated at 50 cc. and 55 cc., and determine the polarization of the acid invert solution at 20°C., multiplying the result by $^{11}/_{10}$ to correct for the dilution.

Calculation of Results.—Since the total volume of water and lead solution added to the defatted chocolate was 110 cc., the apparent per cent. of sucrose is found by the formula

$$S = \frac{a - b}{142.66 - \frac{t}{2}} \times \frac{110}{100}.$$ (See page 258.)

Likewise, the apparent per cent. of lactose is calculated by the formula

$$L = \left[\left(a \times \frac{110}{100}\right) - S\right] \times \frac{66.5}{52.5}$$

[1] Dubois: *Bur. of Chem., Bull.* **137**, p. 98.

where a equals the direct reading, S the apparent per cent. of sucrose as above, and 66.5 and 52.5 the specific rotations of sucrose and lactose respectively. (See page 260.)

These results for sucrose and lactose are not exact on account of the expansion in volume of the solution caused by dissolving the sugars. Hence from the combined apparent percentages of sucrose and lactose calculate the grams of sugar present in the weight of sample taken. Then the true volume $X = 110 + (G \times 0.62)$, where G = total sugar in grams and 0.62 = increase in volume produced by solution of 1 gram of sugar. Hence the true sucrose per cent. $= \frac{SX}{110}$ and the true lactose per cent. $= \frac{LX}{110}$.

The same method may be used in the case of sweet chocolates, but simplified by the absence of lactose.

Notes.—The reason for adding a measured quantity of water rather than making up to the mark in a graduated flask in the usual way, is the large amount of insoluble material in cocoa products, which would give an indeterminate volume of sugar solution by the ordinary method.

The error due to the volume of insoluble material can also be avoided, as suggested by Woy,[1] by using the method of double dilution (page 115).

If a suitable centrifuge is not available the following method[2] may be substituted with equally good results in most cases:

Weigh the normal weight of the finely divided material into an 8-ounce nursing bottle, add 90 cc. of water at room temperature, cork the bottle and place it in a steam-bath for 20 minutes, removing the stopper for a moment at the end of about 5 minutes to relieve the pressure. Twice during the 20-minute period shake the bottle thoroughly so as to emulsify completely the chocolate solution. Remove the bottle from the steam-bath and cool to room temperature, add 10 cc. of basic lead acetate solution, mix thoroughly and filter through a folded filter. On the solution thus obtained determine the sugars as above.

If the per cent. of lactose found is very small the polariscopic

[1] *Z. Nahr. Genussm.*, **1899**, 892.

[2] Bigelow and Albrech: *Bur. of Chem., Bull.* **137**, p. 100.

method would better be checked against determinations by copper, using the Munson and Walker method as described on page 237 and taking the sugar value from the appropriate column for sucrose and lactose mixtures.

There is a small amount of reducing sugar present naturally in the cocoa bean, but the quantity is so small that it can be neglected in the determination of added sugars.

Pentosans.—Follow the method given under General Methods for Carbohydrates, page 264.

Theobromine and Caffeine.[1]—Boil 10 grams of the powdered cocoa or chocolate with 5 grams of magnesium oxide and 300 cc. of water for 30 minutes, using a 500-cc. Erlenmeyer flask with a small funnel in the neck to retard evaporation. Filter by suction on a disc of filter paper in a Büchner funnel. Return the residue and paper again to the 500-cc. flask, add 150 cc. of water and boil for 15 minutes. Filter as before and repeat the 15-minute boiling. Finally filter and wash once or twice with hot water. Evaporate the combined filtrates to dryness, together with about 10 grams of fine sand, in a thin glass or lead-foil dish.

Grind dish and residue to a coarse powder in a covered mortar. If the lead dish was used, strip the residue as much as possible from the dish and grind it as above. Cut the dish into pieces and add to the residue. Transfer all the material to a Soxhlet extracting apparatus and extract with 100 cc. of chloroform for 8 hours. Surround the extractor and flask with a cylinder of asbestos paper so that the extraction may be made by the hot solvent. Distil off the chloroform and dry the flask and residue in the water oven to constant weight. In many cases the residue will be white and consist of nearly pure theobromine and caffeine. If the original material, however, was quite impure, the extracted alkaloids will be brownish.

If it is desired to separate the alkaloids or to purify a discolored residue, treat the weighed residue for several hours at room temperature with 50 cc. of pure benzol or carbon tetrachloride, stirring or shaking frequently. Filter through a small filter, distil off the solvent, boil the residue several times with water, filter and evaporate the aqueous solution of caffeine

[1] Dekker: *Rec. Trav. Chem.*, **1903**, 143; *Chem. Zentr.*, **1903**, 62.

in a weighed dish. The residue insoluble in the benzol or carbon tetrachloride, together with the filter, is treated several times with boiling water, filtered, the solution of theobromine evaporated and dried at 100°C. to constant weight.

If the residue of theobromine obtained in this way is still impure the exact amount may be determined by converting it into the insoluble silver salt by Kunze's method.[1] Dissolve the residue in 150 cc. of water, make slightly alkaline with ammonia and add an excess of tenth-normal silver nitrate solution. (About 12 parts of silver should be added for each part of impure theobromine weighed.) Boil down to 75 cc., add 75 cc. of water, and repeat the boiling. The solution should be perfectly neutral. If it contains the slightest amount of free ammonia, add water and boil until free.

Filter off the insoluble silver substitution product of theobromine, $C_7H_7N_4O_2Ag$, and wash with hot water. To the filtrate add 5 cc. of a cold saturated solution of ferric alum and enough nitric acid, which has been boiled to free it from nitrous compounds and then cooled, to decolorize the liquid. Titrate with tenth-normal potassium sulphocyanate solution to a permanent red color.

From the sulphocyanate titration calculate the amount of silver used to precipitate the theobromine, then from the formula for theobromine and for its silver compound calculate the percentage of the alkaloid present.

Notes.—A portion of the theobromine is not present as the free alkaloid but combined as a glucoside, hence is not extracted by the chloroform until set free by boiling with dilute acid, magnesia or other suitable hydrolyzing agent.

Cocoa Red.—Since the cocoa red is found almost entirely in the cocoa bean itself and not in the shells, its determination has been suggested by Ulrich[2] as a method of showing the presence of shells in cocoa.

Place 1 gram of fat-free dry material, which should be finely powdered, in a 300-cc. Erlenmeyer flask, add 120 cc. of acetic acid (50–51 per cent. strength), connect with a reflux condenser and boil for 3 hours. Cool and dilute to 150 cc. with cold

[1] *Z. anal. Chem.*, **1894**, 1.
[2] *Arch. Pharm.*, **249**, 524.

water; shake well and allow to stand at least 12 hours; filter through a dry filter and treat 135 cc. of the filtrate (corresponding to 0.9 gram of the original substance) with 5 cc. of concentrated hydrochloric acid and 20 cc. of 20 per cent. ferric chloride solution. Heat to boiling and boil 10 minutes under a reflux condenser; cool quickly and transfer to a beaker; after standing at least 6 hours, filter upon a weighed filter, washing the precipitate with hot water until free from iron; dry for 6 hours at 105°C. and weigh. Calculate the per cent. of the insoluble iron compound in the fat-free sample and thence in the original material.

Notes.—The boiling with dilute acid is necessary to decompose the glucoside, in which form the crude cocoa red is present. (See page 303.) By hydrolysis the glucoside yields dextrose. theobromine, caffeine and the true cocoa red, having the formula $C_{17}H_{12}(OH)_{10}$. In its reactions the latter much resembles tannin, forming insoluble compounds with salts of lead, iron and copper, also with gelatin and albumin.

Ulrich found in determinations on eighteen samples, representing eight varieties of cocoa beans, values for the iron precipitate ranging from 5.63 per cent. to 7.88 per cent. with an average of 6.12 per cent.; cocoa shells gave 0.0.

Solubility Tests.[1]—(*a*) *Cold Water Extract.*—Shake 4 grams of material in a 200-cc. flask, filled to the mark with cold water, once an hour for 8 hours, and allow to stand undisturbed for 16 hours; filter. Determine total solids in 25 cc. of the filtrate.

(*b*) *Extract at* 65°*C.*—Mix 4 grams with a little water at 65°C. in a 200-cc. flask; fill the flask to the mark with water at 65°C. and keep at this temperature for 30 minutes, shaking every 5 minutes. Cool quickly, adjust to the mark, mix and filter. Determine total solids in 25 cc. of the filtrate.

(*c*) *Extract at* 100°*C.*—Mix well 4 grams of material with 10–15 cc. of boiling water in a 200-cc. flask, then add actively boiling water slowly and with constant stirring to a point about an inch above the mark, stopper, invert once and immerse in a bath of vigorously boiling water for 3 minutes; cool imme-

[1] Howard and Street: *Ann. Rept. Conn. Expt. Sta.*, **1911**, 109; Booth: *Analyst*, **1909**, 142.

diately in ice water, adjust to the mark, invert once and filter. Determine total solids in 25 cc. of the fitrate.

(*d*) *Relative Sedimentation.*—Mix 0.35 gram of chocolate with a few drops of boiling water in a small beaker, adding a little more water gradually until the chocolate makes a thin paste without lumps, wash into a Hortvet tube (see page 283) with boiling water, the total volume being 35 cc. Invert the tube once and centrifuge for 1½ minutes, allowing 10 seconds for coming to speed and 10 seconds for stopping. Note the volume of the sediment. With sweetened products, correct the result for the amount of sugar present in order to obtain a uniform basis of comparison. Make similar tests on several standard cocoas or chocolates and calculate the relative sedimentation.

Notes.—These methods are of distinct value in testing the claims of the so-called "soluble" cocoas. The treatment with boiling water, especially, will be found in many cases to give interesting information since its result is comparable with that effected by the 3-minute boiling usually prescribed in preparing cocoa for the table. The relative sedimentation test is a measure of the miscibility of the chocolate or cocoa, whether it is dependent on the fineness of the particles, or upon the addition of alkali. It is obviously also an indication of the food value of the preparation.

It should be remembered that the tests are only comparative and should be carried out with due regard for uniformity in the details of the procedure.

Unsweetened chocolate is a relatively insoluble substance, even in boiling water. Tests reported by Street on ten samples showed an average solubility in water at room temperature of 12.53 per cent., at 65°C., 14.04 per cent., and at 100°C., 16.11 per cent. The relative sedimentation ranged from 48 to 60.

Varnishes.—Cakes of sweet or plain chocolate as well as chocolate confectionery, especially the cheaper grades, are frequently coated with some form of varnish in order to improve their appearance and keeping qualities, and to aid in handling them in hot weather. Materials employed for this purpose are shellac and such resins as Peruvian balsam, gum benzoin, and rosin, usually in alcoholic solution. Smith[1]

[1] *Bur. of Chem., Bull.* **132**, p. 58.

has described methods for detecting and identifying the resins when used in chocolate coatings.

Preliminary Tests.—Scrape the surface coating from as large a quantity of chocolate as is available and heat the scrapings on the water-bath for some time with 90 per cent. alcohol. Filter from the undissolved fats, sugar, etc., and apply the following tests to the alcoholic solution:

(*a*) Treat with sodium hydroxide and with lead acetate. Shellac gives a bright violet color with the former, and a violet precipitate with the latter. It should be noted, however, that these color reactions are not given by bleached shellac.

(*b*) Dissolve the dry residue from a portion of the alcoholic solution in acetic anhydride by gentle warming in a small porcelain crucible. After cooling, add a drop or two of 60 per cent. sulphuric acid (sp. gr. 1.53). A violet red color indicates the presence of rosin (Liebermann-Storch-Morawski reaction).

(*c*) Mix a portion of the dry residue from the alcoholic solution with 1 or 2 cc. of a solution of crystallized phenol in carbon tetrachloride (1:2) in a porcelain crucible and allow the vapor from a flask containing bromine dissolved in carbon tetrachloride to fall into the crucible. The presence of rosin is shown by a violet and blue color. Care should be taken to have the residue dry, since water or alcohol interferes with the reaction.[1]

(*d*) Peruvian balsam and gum benzoin are indicated by the fragrance of the alcoholic extract, especially when warmed. If a sufficient quantity of chocolate (a pound or two) is available, benzoic acid may sometimes be sublimed from the alcoholic residue and identified by the tests on page 90.

Quantitative Tests.—To purify the resin for the quantitative tests, extract with hot alcohol as above and evaporate the alcoholic solution to dryness on the water-bath with a liberal quantity of clean sand, stirring with a glass rod toward the end of the evaporation. Wash the sand mixture several times with petroleum ether to remove traces of fat or wax, as well as a large part of the resin, and then with hot water to remove the small amounts of sugar, cocoa red, or other extractive matters which may be present. Finally, treat the mixture with warm dilute sodium carbonate solution, cool and filter. Acidify

[1] Halphen: *J. Pharm. Chim.*, **1902,** 478; *Analyst*, **1903,** 9.

the filtrate, allow the finely divided precipitate of resin to settle out and filter on a Gooch or Büchner filter.

On the purified material determine the iodine, acid, ester and saponification numbers, following the methods described under Edible Oils, pages 157 to 166. If only a limited amount of material is available, use it for the iodine number, since this is the most important. Use 2 grams or more of the sample, dissolve it in warm glacial acetic acid and apply the Hanus method directly.

Note.—The more important values for shellac and rosin are given in the following table:[1]

	Shellac	Rosin
Iodine number	7.5 to 11 (average = 9)	125 (average)
Acid number	55 to 65	125 (average)
Ester number	150 (average)	0 to 6

The low iodine number is especially characteristic as showing the presence of shellac.

Determination of Casein in Milk Chocolate.[2]—Remove the fat from 20 grams of the sample as described on page 309. Rub 10 grams of the dry residue in a mortar, with the gradual addition of 1 per cent. sodium oxalate solution, avoiding the formation of lumps, and pour into a 250-cc. graduated flask until 200 cc. of the sodium oxalate solution have been used. Place the flask on an asbestos board and heat slowly to boiling, taking at least 30 minutes for the heating. During the heating have a funnel, the stem of which has been closed by melting, rest in the neck of the flask. Fill to the mark with hot sodium oxalate solution and allow to stand with frequent shaking until the next day. Fill again to the mark with sodium oxalate solution, mix and filter through a folded filter. To 100 cc. of the filtrate add 5 cc. of a 5 per cent. solution of uranium acetate and then drop by drop, with constant stirring, 35 drops (approximately 1.5 cc.) of 30 per cent. acetic acid. Separate the precipitated casein by centrifuging and wash three times in the centrifuge with 75-cc. portions of a solution containing 5 grams of uranium acetate and 3 cc. of 30 per cent. acetic acid in 100 cc. Finally transfer

[1] Parry: Allen's Com. Org. Anal., 4th Ed., Vol. IV, p. 70.

[2] Baier and Neumann: *Z. Nahr. Genussm.*, **1909**, 13.

the precipitate to a nitrogen-free filter by means of the washing solution and determine the nitrogen by the Kjeldahl method, page 25. Multiply the result by 6.38 to obtain the casein.

Note.—From the determination of casein thus made the protein content of the added milk may be calculated by multiplying by 1.25. (See page 106.)

Determination of Milk Fat.—Weigh out 5 grams of the dry extracted fat (conveniently obtained from the petroleum ether extract in the determination of sugars, page 310) and determine its Reichert-Meissl number as described on page 204. Assuming 28 as an average value for the Reichert-Meissl number of butter fat and 0.5 as the corresponding value for cocoa butter, the calculation of the amount of butter fat is evident from the following equation, in which X and Y represent respectively the grams of butter fat and of cocoa butter present in the 5 grams taken for analysis, and A the Reichert-Meissl value obtained:

$$\frac{28}{5}X + \frac{0.5}{5}Y = A$$
$$X + Y = 5$$

By solving for X, knowing the percentage of total fat in the chocolate, the per cent. of milk fat is readily determined.

Note.—From the determinations of fat, casein and lactose, thus made, the approximate per cent. of milk solids in the chocolate may be found.

For calculation of the fat in a legal case, however, the Reichert-Meissl value should be taken as 24 rather than 28, the former being the minimum value permissible by the Federal standard for butter fat.

Examination of the Cocoa Fat.—For the detection of cocoa-butter substitutes, the determination of the iodine number, saponification number, melting point and refractive index will usually suffice. These may be carried out as described under Edible Fats and Oils, pages 150 to 165. The Reichert-Meissl number may prove helpful, and if cocoanut oil is suspected, the methods suggested for its detection in butter fat, page 219, will be of service. Conclusive evidence of the presence of animal fats, as tallow, may be obtained by the phytosteryl acetate test, page 176.

A preliminary test which is often used for the detection of wax or tallow is Björklund's ether test,[1] carried out as follows:

Place about 3 grams of the fat in a test-tube, add 6 grams of ether, previously cooled to 18°C., cork the tube and shake. The fat should dissolve to a clear solution. Then immerse the tube in water at 0°C., and note the number of minutes required for the liquid to become milky, or to deposit white flocks. Note also the temperature at which the solution becomes clear again when removed from the water. The following figures are given by Björklund to show the value of the method:

	Turbidity at 0°C. after minutes	Clear solution at 0°C.
Cocoa butter	10–15	19–20
Cocoa butter and 5 per cent. beef tallow	8	22
Cocoa butter and 10 per cent. beef tallow	7	25

Lewkowitsch[2] recommends that in carrying out this test observation be made also of the characteristic way in which genuine cocoa butter crystallizes as compared with adulterated samples. In the former case tufts of distinct crystals appear on the bottom and sides of the tube, while with 5 per cent. or more of tallow flocks separate from the cooled solution.

Microscopical Examination.—The principal microscopical characteristics of cocoa are summarized in Chapter II. From the view point of detecting adulterants the most important tissues of the bean are the small, nearly circular starch grains, which exhibit a tendency to gather in groups of two or three; the angular polygonal cells of the cotyledons, which are rather easily broken up; and the masses of yellow, brown or even violet pigment. These are shown in Fig. 90, page 499.

The most prominent elements of the powdered shell are the spiral vessels, shown in fragments at *b*, and in large masses at *a*, Fig. 91. Since it is impossible to separate the shell from the cotyledon absolutely in the process of manufacture, occasional fragments of the spiral vessels, which are by far the most numerous distinctive elements of the shell, will be found in genuine powdered cocoa. The occasional occurrence of these should be disregarded, but the constantly recurring presence of them in

[1] *Z. anal. Chem.*, **1864**, 233.

[2] Oils, Fats and Waxes, 5th Ed., Vol. II, p. 591.

large numbers, especially of whole spirals or of bundles of vessels as at *a*, should be taken as evidence of adulteration with shell powder. Other tissues of the shell, as the epidermal hairs, or "Mitscherlich bodies," and thin epidermal cells, although of interest in studying the structure of the bean, on account of their scarcity, or fragile nature, are not present in the roasted and ground material in sufficient quantity to be of much analytical importance.

Foreign starches, of which the most common in cocoa are wheat, sago and arrowroot, are distinguished by their characteristics of shape, size and markings, as detailed in Chapter II.

For microscopical examination, especially in the case of chocolate, a part of the sample should be treated on a filter with several portions of ether, dried and powdered. The tissues of the shell may be rendered more apparent by heating the material gently with chloral hydrate as described on page 35.

Interpretation of Results

Composition of Cocoa Beans and Cocoa Shells.—Extended analyses of shelled cocoa beans (cocoa nibs) and of the shells have been made, following modern methods of analysis, by Winton[1] and by Booth.[2] These are summarized in Tables XLIX and L.

In these analyses, which show in general the maximum variation in composition which may be expected in pure unsweetened chocolate, should be noted especially the ash, ash insoluble in acid and crude fiber, as compared with the corresponding values for shells, and the starch (both by acid and by diastase), which serves to detect such starchy adulterants as wheat flour or arrowroot. Both shells and foreign starches are, of course, more readily detected qualitatively in the microscopical examination, the above determinations aiding in showing the extent of adulteration.

Cocoa shells are much less uniform in composition than the shelled beans, especially as regards the percentages of ash and

[1] Winton, Bailey and Silverman: *Ann. Rept. Conn. Agr. Expt. Sta.*, **1902**, 270.

[2] Booth, Cribb and Richards: *Analyst*, **1904**, 134.

TABLE XLIX.—ANALYSES OF AUTHENTIC COCOA BEANS AND SHELLS (WINTON)

Determination	Shelled cocoa beans						Shells					
	Air-dry sample			Water- and fat-free sample			Air-dry sample			Water-and fat-free sample		
	Max. per cent.	Min. per cent.	Av.[1] per cent.	Max. per cent.	Min. per cent.	Av. per cent.	Max. per cent.	Min. per cent.	Av. per cent.	Max. per cent.	Min. per cent.	Av. per cent.
Water	3.18	2.29	2.72	.....	.....	.....	6.57	3.71	4.87	.....	.....	.....
Ash (total)	4.15	2.61	3.32	8.81	5.76	7.04	20.72	7.14	10.48	21.97	5.63	11.33
Soluble ash	1.86	0.73	1.16	3.96	1.60	2.46	5.67	2.02	3.67	6.11	2.16	3.97
Ash insoluble in acid (sand)	0.07	0.00	0.02	0.14	0.00	0.05	11.18	0.05	2.51	11.86	0.05	2.70
Alkalinity of ash (cc. N/10 acid for 1 gm. of sample)	3.35	1.50	2.51	7.12	3.29	5.32	5.92	5.02	5.52	6.47	5.32	5.97
Theobromine	1.32	0.82	1.04	2.92	1.66	2.21	0.90	0.20	0.49	0.97	0.22	0.52
Caffeine	0.73	0.14	0.40	1.55	0.31	0.86	0.28	0.04	0.16	0.31	0.04	0.17
Crude fiber	3.20	2.21	2.64	6.56	4.70	5.61	19.21	12.93	16.63	20.72	13.71	18.01
Starch by acid hydrolysis	12.37	9.30	11.16	25.68	19.80	23.66	13.89	9.87	11.62	15.42	10.47	12.59
Starch by diastase	8.99	6.49	8.07	18.61	13.82	17.10	5.16	3.36	4.14	5.59	3.65	4.47
Total nitrogen	2.54	2.20	2.38	5.41	4.74	5.05	3.17	1.74	2.34	3.41	1.87	2.54
Fat	52.25	48.11	50.12	.....	.....	.....	5.23	1.66	2.77	.....	.....	.....
Constants of fat (ether ext.)												
Melting point, °C.	35.0	32.3	33.3	.....	.....	.....	.....	.....	.....	.....	.....	.....
Refractive index, 40°C.	1.4579	1.4565	1.4573	.....	.....	.....	.....	.....	.....	.....	.....	.....
Iodine number	37.89	33.74	34.97	.....	.....	.....	.....	.....	.....	.....	.....	.....

[1] In each case the average represents 17 varieties.

TABLE L.—ANALYSES OF NIBS AND SHELLS FROM KNOWN SOURCES (BOOTH)

Nibs	Ash, per cent.	Soluble ash, per cent.	Ash insol. in HCl, per cent.	Alkalinity as K_2O, per cent.	Cold water extract, per cent.	Nitrogen, per cent.	Fat, per cent.	Crude fiber, per cent.
African	2.52	0.98	0.05	0.38	11.8	1.84	50.2	.....
Grenada	2.60	1.04	0.03	0.55	9.8	2.26	50.8	2.94
Guayaquil	3.16	1.32	0.04	0.53	11.4	.....	.....	.....
Trinidad	2.73	0.95	0.00	0.44	12.0	2.32	55.7	2.48
Caracas	3.24	1.58	0.08	0.74	.....	.....	.....	.....
Bahia	2.68	1.22	0.05	0.51	9.5	1.98	44.4	.....
Accra	3.22	1.36	0.04	0.41	11.4	2.46	50.6	2.87
Ceylon	3.81	1.66	0.03	0.67	11.9	2.44	50.2	2.36
Puerto Cabello	3.88	1.50	0.13	0.64	12.6	2.35	51.3	3.02
Shells								
Ceylon	6.61	4.78	1.00	2.54	20.7	2.40	3.1	12.80
African	5.63	3.53	1.79	2.63	20.4	2.91	3.5	15.70
Guayaquil	8.19	5.25	1.45	3.36	24.6	2.13	5.9	12.85
Puerto Cabello	20.82	5.24	8.33	1.13	23.5	.....	5.7	13.83

sand, since they are contaminated by variable amounts of adhering dirt and are more affected by the heat of roasting. An instance is seen in the figures for Puerto Cabello shells in Booth's table above.

Calculated to the water- and fat-free basis, which, on account of the great variation of these constituents in different cocoa products, is the only fair basis for comparison, it is seen that the minimum value for crude fiber in the shells is 13.71 per cent. and the maximum for shelled cocoa is only 6.56 per cent., from which it is evident that the addition of each 15 per cent. of shells increases the crude fiber by at least 1 per cent.

It should be noted also that the soluble ash and alkalinity of the ash are both higher in the shells, hence the addition of the latter would increase these values in genuine cocoa, a fact which should be taken into consideration in using these values to judge whether a sample has been treated with alkali. (See page 328.)

Standards for Cocoa Products.—The Federal standards for chocolate and its preparations are as follows:[1]

[1] *U. S. Dept. Agr., Office of the Secretary, Circ.* **19**; Food Inspection Decision 136.

Chocolate, plain chocolate, bitter chocolate, chocolate liquor, bitter chocolate coatings, is the solid or plastic mass obtained by grinding cocoa nibs without the removal of fat or other constituents except the germ, and contains not more than 3 per cent. of ash insoluble in water, 3.50 per cent. of crude fiber, and 9 per cent. of starch, and not less than 45 per cent. of cocoa fat.

Sweet chocolate, sweet chocolate coatings, is chocolate mixed with sugar (sucrose), with or without the addition of cocoa butter, spices or other flavoring materials, and contains in the sugar- and fat-free residue no higher percentage of either ash, crude fiber, or starch than is found in the sugar- and fat-free residue of chocolate.

Cocoa, powdered cocoa, is cocoa nibs, with or without the germ, deprived of a portion of its fat and finely pulverized, and contains percentages of ash, crude fiber, and starch corresponding to those in chocolate after correction for the fat removed.

Sweet cocoa, sweetened cocoa, is cocoa mixed with sugar (sucrose), and contains not more than 60 per cent. of sugar (sucrose), and in the sugar- and fat-free residue no higher percentage of either, as crude fiber or starch, than is found in the sugar- and fat-free residue of chocolate.

Milk chocolate and *milk cocoa* should contain not less than 12 per cent. of milk solids, and the so-called nut chocolate should contain substantial quantities of nuts.

The standard for plain chocolate proposed by Whymper[1] at the Berlin Congress of Cocoa and Chocolate Makers in 1911, requires not less than 45 per cent. of cocoa fat; not more than 4 per cent. ash (ratio of soluble to insoluble ash not to exceed 2:3); not more than 6 per cent. moisture; not more than 2.75 per cent. pentosans; not more than 13 per cent. cocoa starch.

Booth[2] has proposed as the result of numerous analyses of milk chocolates that it should be defined as a "preparation composed exclusively of roasted, shelled cocoa beans, sugar, and not less than 15 per cent. of the dry solids of full-cream milk, with or without a small quantity of harmless flavoring matter."

[1] Cocoa and Chocolate: Their Chemistry and Manufacture, p. 311.
[2] *VIIth Int. Cong. of Appl. Chem.*, VIIIc, 178.

Plain Chocolate.—The composition of pure unsweetened chocolate, as sold, naturally resembles closely that of cocoa nibs as tabulated above. There is, however, somewhat less variation in composition since the product as sold is ordinarily prepared from a blend or mixture of cocoa beans, and is consequently more uniform. This is shown by the following figures. for ten well-known brands examined in 1911.[1]

TABLE LI.—ANALYSES OF PLAIN CHOCOLATE

Determination	Maximum per cent.	Minimum per cent.	Average per cent.
Total ash	3.76	2.91	3.40
Soluble ash	1.66	1.11	1.42
Ash insoluble in acid	0.21	0.03	0.10
Alkalinity of ash (cc. N/10 acid for 1 gram.)	4.42	3.29	3.96
Fat	52.35	47.05	49.56
Nitrogen	2.46	2.09	2.29
Soluble in cold water	14.00	11.02	12.53
Soluble in water at 65°C	15.68	12.48	14.04
Soluble in water at 100°C	17.64	14.38	16.11
Relative sedimentation	60	45	52
Iodine number of fat	36.99	34.15	35.32
Refractive index of fat, 40°C	1.4572	1.4563	1.4569

In Table LII are given several similar analyses of adulterated plain chocolate.[2] Samples 1 and 2 contained added starch and in sample 3 part of the cocoa butter was replaced by cocoanut oil. In two of the samples there is a deficiency of fat.

Apart from the presence of foreign starches, the adulterants to be looked for especially in plain chocolate are the addition of shells and of foreign fats. The former is discussed under cocoa powder on page 327. The use of cocoa butter substitutes is possibly on the increase because of the great demand at present for the smooth, soft chocolate which must be made by finer grinding and by the addition of cocoa butter to the cocoa mass.

The substitutes most commonly employed include the following fats, either alone or in combination: Cocoanut oil stearine,

[1] Street: *Ann. Rept. Conn. Expt. Sta.*, **1911,** p. 103.

[2] Winton: *Ann. Rept. Conn. Expt. Sta.*, **1903**, p. 123.

TABLE LII.—ANALYSES OF ADULTERATED CHOCOLATE

Determination	1	2	3
Total ash	3.02	2.96	3.96
Soluble ash	1.33	1.25	1.53
Ash insoluble in acid	0.07	0.08	0.14
Alkalinity of ash (cc. N/10 acid for 1 gram.)	1.65	1.85	1.95
Fat	40.69	46.48	44.61
Nitrogen	2.42	1.99	2.51
Crude starch	21.60	19.11	14.00
Pure starch	17.64	15.03	10.53
Crude fiber	2.63	2.42	3.64
Melting point of fat, °C.	31.75	31.50	29.50
Refractive index of fat at 40°C.	1.4572	1.4569	1.4548
Iodine number of fat	37.83	37.46	26.05

palm nut stearine, tallow and cotton seed stearine. Vegetable oils, such as peanut and sesame, and even paraffin and beeswax, have been reported. The analytical constants of most of these have been included in Table XXIX on page 175, but for convenience the following values, taken from an extended table by Whymper,[1] are given here.

TABLE LIII.—CONSTANTS OF COCOA BUTTER SUBSTITUTES

Fat or oil	Specific gravity at °C.	Melting point, °C.	Saponification value	Reichert-Meissl number	Iodine value
Cocoa butter	0.964–0.974 at 15	30.0–34.0	192–195	0.2–0.9	32.0–42.0
Cottonseed stearine	0.867 at 100	27.0–45.0	194.5	0.8–1.0	89.0–93.0
Cocoanut oil	0.9259 at 15	20.0–28.0	246–252	6.6–8.4	8.0– 9.0
Cocoanut stearine	0.8700 at 100	29.3–29.5	252	3.4	4.0– 4.5
Palm nut oil	0.8731 at 99/15.5	23.0–30.0	243–255	5.0–6.8	10.5–17.5
Palm nut stearine	0.8700 at 100	31.5–32.0	242	2.2	8.0
Tallow	0.925–0.940 at 15.5	38.0–50.0	193–198	0.2	33.48
Peanut oil	0.911–0.926 at 15.5	.....	186–194	0.5	83.0–101.0
Sesame oil	0.921–0.925 at 15.5	.....	188–193	1.2	109.0–112.0
Paraffin	0.824–0.940 at 15.5	36.7–58.3	0.0	0.0	3.9–4.0

Such substitutes as cocoanut and palm oil stearines, which are sold in the trade under such names as "Vegetine," "Vegetable butter," "Laureol" and "Palmin," will be shown by their high saponification value and low iodine number. In the absence of

[1] *Loc. cit.*

butter fat, the increased Reichert-Meissl number will also be indicative. The change in melting point may be noticeable, but it is hardly as decisive as the constants mentioned. The specific methods for cocoanut oil may be employed (see page 219), but would not be of so much value in the case of the cocoanut stearine. The liquid vegetable oils lower the melting point, especially of the fatty acids, and raise the iodine number and refractive index, without affecting to any great degree the saponification value and Reichert-Meissl number. It should be remembered in this connection that cocoa butter obtained from cocoa shells may have iodine values one or two units higher than the ordinary. Paraffin or beeswax, the addition of which is comparatively rare, would be detected by the lowered saponification value and the presence of much unsaponifiable matter. Tallow would raise the melting point and the refractive index while the other constants remained practically unchanged. Indications of its presence would be given also by Björklund's test or the phytosteryl acetate test.

Sweet Chocolate.—This is, of course, only plain chocolate to which a certain proportion of sugar and flavoring, usually vanilla, has been added, and is defined as such in the standards given on page 323. Naturally, the same forms of adulteration that have been considered under plain chocolate would apply here, with the added possibility that excessive amounts of sugar may be present. Although no definite limit is given in the standard for the amount of sugar that may be added, the maximum standard of 60 per cent. adopted for sweetened cocoa, would seem to be a reasonable requirement for sweet chocolate. After correcting for the sugar present, the analysis of sweet chocolate should conform to the figures given for plain chocolate on page 324, and to the standards which have been formulated for the latter.

Typical analyses of sweet chocolates are given in Table LIV. The first three are analyses of well-known brands, No. III, the "Dot" chocolate made by the Walter Baker Co., being noticeably lower in sugar content than is usual with sweet chocolate, and for that reason being preferred by some. IV and V are adulterated samples, the adulterant in each case being foreign starch. The percentage of starch in a pure sweet chocolate would not ordinarily exceed 5.0 per cent., or 10.5 per cent. in the sugar-free material.

VI, although sold as "powdered chocolate," is not a chocolate at all, but a sweetened cocoa, since a large part of the fat has been removed.

TABLE LIV.—ANALYSES OF SWEET CHOCOLATE

Determination	I	II	III	IV	V	VI
Total ash (per cent.)	1.34	1.26	1.68	1.04	0.88	2.18
Ash soluble in water (per cent.)	0.69	0.92	0.78	0.71	0.53	0.88
Alkalinity of ash, cc. N/10 acid for 1 gram	1.55	1.51	2.06	1.40	1.22	2.22
Fat (per cent.)	27.90	31.78	41.19	25.90	27.23	11.22
Nitrogen (per cent.)	0.90	0.63	1.21	1.07	0.89	1.61
Starch (acid hydrolysis) (per cent.)	.....	.....	.....	11.60	9.17	.....
Sucrose (per cent.)	55.09	53.01	34.55	48.02	56.21	55.53
In fat-and sugar-free material						
Total ash (per cent.)	7.87	8.28	6.93	3.99	5.32	6.86
Nitrogen (per cent.)	5.28	4.14	5.00	4.10	5.46	4.84
Starch (per cent.)	.....	.....	.....	44.45	55.42	.....
Soluble in cold water (per cent.)	25.45	39.78	27.08	.....	.....	27.22

Cocoa.—On account of the removal of a part of the fat, the other constituents will be distinctly higher in the case of powdered cocoa than in chocolate. Calculated, however, to a fat-free basis, they ought to agree with the analyses of roasted cocoa nibs stated on the same basis and given on page 321. The first four analyses in Table LV on page 330 are typical of unadulterated powdered cocoa as found on the market.

Detection of Shells.—Probably the easiest method of detecting shells is by microscopical examination. This method, however, is entirely qualitative and gives no idea of the proportion of shells present, so that help must often be had from the chemical examination. The greatly increased content of ash and crude fiber in cocoa shells as compared with the shelled beans has already been noted on page 322 in connection with the analyses given in Table XLIX. Of the two determinations, greater weight should be laid upon the fiber, but even in this case it is doubtful, on account of the variations in different samples of cocoa beans and sshell, whether the addition of much less than 25 per cent. of shells could be declared with certainty. If the general characteristics of the cocoa powder to which addition of shells was suspected were

known, closer results than this could be obtained. Ludwig,[1] for example, determined the crude fiber (by a somewhat different method) in six cocoas ranging from 4.98 per cent. to 5.96 per cent. (average = 5.60 per cent.), calculated on the fat-free material. When these were mixed in equal proportion, the addition of 10 per cent. of cocoa shells, containing 14.47 per cent. of crude fiber, increased the amount present to 6.16 per cent.

Of possibly greater value, although the determination is tedious, is the estimation of pentosans. Adan[2] reports from 1.19 per cent. to 2.19 per cent. of pentosans in cocoa nibs, as against 7.57 per cent. to 10.53 per cent. in shells; Luhrig and Segin[3] find 2.51–4.58 per cent. in nibs and 7.59–11.23 per cent. in the corresponding shells. Many other observers have reported similar results.

Ulrich[4] as the result of a critical study of the more important methods for showing the addition of cocoa shells, recommends the estimation of cocoa red, by the method described on page 313, as the most satisfactory of the chemical methods, it showing the presence of 10 per cent. or more. Sample VII in Table LV, page 330, is adulterated with cocoa shells.

Detection of Added Alkali.—The addition of alkali, in cocoas treated by the so-called Dutch process, will usually be indicated by the increased proportion of soluble ash and the high alkalinity of the ash, especially of the soluble portion. This is clearly shown in the following comparison of forty-four samples of untreated cocoa and eight samples of alkali-treated cocoa made at the Conn. Agr. Expt. Station in 1911. It should be noted that the alkalinity of the ash, as given in the table, refers to the alkalinity of the total ash, not of the soluble portion. This would be distinctly lower, and with untreated cocoa, will seldom exceed 3.75 cc. of tenth-normal acid for 1 gram of sample. It is required under the Federal Food Law[5] that cocoa which has been treated with an alkali or alkaline salt so as to increase the mineral matter present, should bear a label stating the fact that mineral

[1] *Z. Nahr. Genussm.*, **1906,** 153.
[2] *Bull. Soc. Chem. Belg.*, **1907,** 211.
[3] *Z. Nahr. Genussm.*, **1906,** 161.
[4] *Loc. cit.*
[5] Food Inspection Decision 136.

ingredients have been added and the amount. Cocoas and chocolates containing an appreciable amount of free alkali are held to be adulterated.

Determination	Untreated cocoa			Alkali-treated cocoa		
	Max. per cent.	Min. per cent.	Average per cent.	Max. per cent.	Min. per cent.	Average per cent.
Total ash	6.08	4.33	5.25	9.25	5.96	7.49
Water-soluble ash	3.15	1.72	2.32	7.62	4.53	5.79
Alkalinity of ash, cc. N/10 acid for 1 gram	6.85	4.90	6.04	12.29	7.29	9.36

In Table LV, page 330, analyses V and VI are typical Dutch process cocoas.

"Soluble" Cocoa.—Although packages of cocoa are frequently labeled "perfectly soluble" or "pure soluble cocoa," there is in reality no such thing as a "soluble" cocoa. In any case the larger part of the powder is insoluble in water and in those brands which claim to be "soluble," due usually to treatment with alkali, it will be found that while some of them do not settle as readily from their suspension in water as do untreated cocoas, the difference can be ascribed to the fineness of the powder fully as much as to the treatment with alkali. The figures given below, taken from the work of the New Haven Experiment Station are interesting in this connection.

Determination	Average of 44 straight cocoas per cent.	Average of 8 "soluble" cocoas (alkali-treated) per cent.
Soluble in cold water	19.34	19.75
Soluble in water, 65°C.	21.55	21.33
Soluble in water, 100°C.	23.29	24.99
Organic matter soluble in cold water	17.02	13.96
Relative sedimentation	96.0	87.0
Ash soluble in cold water	44.0	76.0

These figures show that while 1.7 per cent. more of the total cocoa is soluble in boiling water, over 3 per cent. less of organic matter is soluble in cold water, that is, the apparently slightly increased solubility is due to the added amount of alkaline

salts and not to any change in the cocoa mass itself. On the whole, the "soluble" cocoas show a lower water-solubility of the *cocoa mass* than do the others. The difference in relative sedimentation is only slight.

In other words, the use of the word "soluble" on cocoa as sold at present is a form of misbranding. To quote again from Food Inspection Decision 136: "In the opinion of the Board, cocoa not treated with alkali is not soluble in the ordinary acceptance of the term. Cocoa before and after treatment with alkali shows essentially the same lack of solubility. To designate the alkali-treated cocoa as 'soluble' cocoa is misleading and deceptive."

TABLE LV.—ANALYSES OF TYPICAL COCOA POWDERS

Determination	I per cent.	II per cent.	III per cent.	IV per cent.	V per cent.	VI per cent.	VII per cent.
Total ash	5.07	4.67	4.65	4.52	7.98	6.63	6.84
Ash soluble in water	2.02	1.95	1.81	1.76	6.46	5.09	3.32
Alkalinity of ash, cc. N/10 acid	2.40	2.38	2.20	2.14	5.00	8.32	3.13
Crude fiber	3.80	4.26	3.77	3.66	4.39	3.72	10.15
Starch by acid hydrolysis	15.87	16.57	13.93	14.80	13.71	13.98	8.12
Starch by diastase	11.29	12.40	9.16	10.79	9.35	9.16	3.81
Nitrogen	3.50	3.27	3.15	3.32	3.33	3.04	2.12
Fat	26.22	29.14	34.51	29.55	28.99	29.20	6.82
Theobromine and caffeine	1.39	1.32	1.21	1.25	1.03	1.28	0.65

The analysis of sweetened cocoa needs no discussion, since apart from the sugar content, which should not exceed 60 per cent., it conforms to the composition of unsweetened cocoa.

Milk Chocolate.—From the ratio between the milk fat and the lactose, determined as described on pages 318 and 310, it can be seen whether the milk used was whole or partially skimmed milk. Comparison may be made with the same ratio calculated from the figures given in Table XIII for the composition of pure milk. From the formulæ given on page 134, the approximate percentage of milk solids present may be calculated from the fat and lactose, or the protein may be calculated directly from the determination of casein, in which case all of the milk solids, with the exception of the ash, are available.

Analyses of milk chocolate are given by Booth[1] and by Street.[2] Since the latter represent the brands sold on the American market the typical examples given below are taken largely from that source.

TABLE LVI.—TYPICAL ANALYSES OF MILK CHOCOLATE

Determination	I, per cent.	II, per cent.	III, per cent.	IV, per cent.	V, per cent.	VI, per cent.
Total ash	1.56	1.85	1.71	1.67	1.79	1.16
Ash soluble in water	0.54	0.63	0.66	0.85	0.82	0.40
Alkalinity of ash, cc. N/10 acid for 1 gram	1.19	1.82	2.09	1.93	2.10	0.86
Nitrogen	1.17	1.36	1.19	1.11	1.42	.....
Sucrose	48.31	45.81	43.09	49.45	39.45	48.88
Lactose	7.28	7.75	3.57	2.25	6.24	1.12
Fat	29.95	28.69	32.13	28.77	33.23	37.02
Reichert-Meissl number of fat	6.2	5.9	5.0	3.2	4.1	8.8
Butter fat (calculated)	7.72	7.06	6.68	3.82	5.68	13.09

It is clearly shown by inspection of the values for lactose and fat in the table that in every case milk distinctly above the average in quality was employed. Sample VI, which was an imported chocolate, analyzed in the author's laboratory, was labeled "Cream Chocolate" and the analysis confirms this. It is hardly necessary to point out that neither the lactose nor the butter fat alone is evidence of the character of the milk used, but the ratio between the two must be considered. Sample V shows a higher content of butter fat than does Sample IV, but the milk used was of inferior quality. The actual amount of milk solids present should, of course, be taken into account also.

Selected References

EWELL.—Analyses of Cocoa Preparations. U. S. Dept. of Agr., Bur. of Chem., Bull. 13, Part VII.

JACOUTOT.—Chocolate and Confectionery Manufacture.

WHYMPER.—Cocoa and Chocolate.

WINTON, SILVERMAN AND BAILEY.—Authentic Analyses of Cocoa. Ann. Rept. Conn. Agr. Expt. Station, 1902, 1903.

ZIPPERER.—The Manufacture of Chocolate and Other Cocoa Preparations.

[1] *Analyst*, **1909**, 146.

[2] *Ann. Rept. Conn. Agr. Expt. Sta.*, **1911**, 106.

CHAPTER VIII

SPICES

Spices are of especial interest to the student of food analysis, not only because they afford perhaps the best opportunity for the happy combination of chemical and microscopical tests, but also because from their nature they are far more likely to be adulterated than would be imagined from their actual monetary value.

The microscopical examination, as explained more fully in Chapter II, is of the greatest value in pointing out quickly and qualitatively the character of the adulterants present, while the chemical tests may serve to confirm the indications of the microscope as well as to show some forms of adulteration, such as partially exhausted or improperly cleaned spices, which are not revealed by the microscope.

The general adulterations of spices consist largely in the addition of such inert, refuse materials as ground nut-shells, sawdust, bark, fruit stones and roasted cereals. Cheaper spices may be substituted in part for the more costly ones, as allspice for cloves, or small quantities of the more pungent spices as cayenne, may be employed to conceal by their stronger taste the addition of diluents, as starches. Such adulterations as the substitution of inferior grades of the same spice, the removal of a portion of the volatile oil by steam distillation, or the presence of an excessive amount of sand and dirt, either adhering to the spice through lack of thorough cleaning or added with fraudulent intent, are shown best by chemical methods.

Certain general methods, applicable to all of the spices, will first be described, followed by a more detailed discussion of several of the more important individual spices.

General Analytical Methods

Often the sample of spice will be sufficiently fine to examine without further preparation. Any that show an excessive

amount of coarse particles should be ground until they will pass a sieve having round holes 1 mm. in diameter or one having 60 meshes to the inch.

Moisture.—Weigh 2 grams of the sample and dry it in an air-bath at 110°C. to constant weight, which usually requires about 12 hours. From the total loss in weight deduct the percentage of volatile oil, determined as described below; the difference is the moisture.

Note.—The above method has been adopted as a provisional method by the Association of Official Agricultural Chemists, but it should be noted that with spices containing a considerable proportion of volatile oil the method is not exact.

Ash and Alkalinity of Ash.—Use 2 grams and determine the total, water-soluble and acid-insoluble ash as described under General Methods, page 16.

Ether Extract.—*Total Extract.*—Weigh out 2 grams of the sample and extract it for 16 hours with anhydrous ethyl ether. Some form of continuous extraction apparatus, such as the Soxhlet or Johnson, should be used. For the reasons given on page 23, the latter form of apparatus will be found preferable. Note carefully the precautions there given in the description of the method.

After the extraction allow the ether to evaporate spontaneously at room temperature, then place the flask or dish in a desiccator over fresh, concentrated sulphuric acid for at least 12 hours and weigh.

Non-volatile Extract.—Heat the flask containing the weighed ether extract very gradually to 100°C., taking several hours to reach that temperature. Finally heat at 110°C. to constant weight. The residue is the non-volatile ether extract.

Volatile Extract.—This is the difference between the total and the non-volatile ether extract just determined.

Notes.—The precautions as to the rate and temperature of heating are to avoid loss of volatile oil by oxidation or volatilization at too high a temperature.

The determination of ether extract is, on the whole, the most important in determining the purity or value of a spice, since in nearly every case the pungency or essential quality of the spice is due to ingredients soluble in ether. This may be mainly a

volatile oil, as in cloves, in which case the volatile ether extract would be of most importance, or to some non-volatile substance, like the *capsaicin* of cayenne, which would appear in the residual portion of the ether extract.

Crude Fiber.—Wash 2 grams of the sample on a filter with several portions of ordinary ether, or use the extracted residue from the determination of ether extract. In either case proceed as directed on page 269.

Notes.—The determination of crude fiber is of importance in examining spices, since the adulteration consists frequently in the addition of waste or refuse material derived from the spices themselves or other food products. This material is frequently the outer cellular layer or protective coating of the plant, designed to protect the softer tissues which constitute the edible portion, and as such contains more of the hardened or lignified cells, which means higher values for the crude fiber. This is apparent from the following tabulation of the approximate values for crude fiber in some common spices and adulterants:

Material	Per cent. of crude fiber
Cloves	7–9
Mustard	2.5–3.5
Mustard hulls	10–20
Pepper	10–18
Pepper shells	25–30
Nutshells	50–55
Sawdust	50–60
Olive stones	55–60
Buckwheat hulls	45

It is worth noting in this connection that the residue on the Gooch crucible in the crude fiber determination is excellent material in which to find stone cells or other hard tissues for the microscopical examination. A bit can be taken out from the moist residue on the tip of a knife and transferred to a slide for examination without interfering appreciably with the determination.

Starch.—The method to be followed depends upon the character of the material and the accuracy desired. If the starch content is high and there is comparatively little material which

would yield copper-reducing substances upon hydrolysis, the method by direct acid hydrolysis will be satisfactory. In the case of such spices as white pepper and ginger this method could be used with good results.

On the other hand, with such spices as cloves and mustard, where there is practically no starch, but a relatively large percentage of other copper-reducing substances, either directly or after acid hydrolysis, erroneous conclusions may be drawn unless the diastase method be employed.

It may be said, however, that on account of the greater ease and rapidity of the direct conversion method it is generally employed, due consideration being given to the high results found with some of the spices and the presence of foreign starch reported only when confirmed by microscopical examination. In doubtful cases, or if legal action may result from the conclusions of the analyst, the diastase method should be employed.

In any case, use about 4 grams of the sample, wash with ether, then with alcohol, and proceed as directed on page 263.

Pepper

Black pepper is the dried fruit of *Piper nigrum*, a perennial climbing shrub indigenous to the forests of India, but now extensively cultivated throughout the East Indies. The small fruit grows loosely on pendulous spikes, of which twenty to thirty, each containing twenty to forty berries, are produced by a single vine. The pepper berry is a small, round, sessile, fleshy fruit, which first appears green, then red, and finally yellow when ripe.

The berries are gathered when beginning to turn red, partially dried on the ground or on mats during several days, then rubbed from the stalks with the hands. When the berries have become thoroughly dried they are shriveled and turn very dark brown or black.

White pepper is not the product of a separate plant, but is the ripened fruit of the black pepper vine, the changed appearance being due to its method of preparation. The berries are soaked in water or buried in damp soil, and after some days

will swell and burst the outer husk, which is then easily removed by rubbing with the hands while the berries are drying in the sunshine, leaving the inner white portion of the berry. Another way is said to be by placing the black pepper in a solution of chloride of lime to remove the dark coating, after which it is rubbed and dried as above.

There are in all about forty different species of the pepper plant, but not all are of commercial importance. The varieties which enter into commerce are usually named from the city or country of export, as Tellicherry, Malabar, Penang and Singapore. Acheen and Lampong pepper come from the west and east coasts of Sumatra, respectively.

Shot pepper is the heavier grades of black pepper put through a soaking and hardening process which gives it a better appearance and brings for it a higher price.

General Composition.—Pepper contains, in addition to starch, which is present in considerable amount, a small quantity of volatile oil, two characteristic alkaloids, *piperin* and *piperidin*, and slight percentages of gum and resin.

The pungency of pepper is due probably in great part to the alkaloid and the resin, modified to a certain extent by the volatile oil.

Analyses of Genuine Pepper.—Many analyses of authentic samples of pepper will be found in the literature. Some of them are obviously incomplete and many of the older ones, being made by inexact methods, possess only historical value. For this reason it seems best to give here instead of a general summary of the analyses, thus including those of doubtful value, only two series (see Table LVII) made by modern methods and representing the commercial varieties found on the American market.

Another excellent series of analyses has been made by Doolittle,[1] comprising 45 samples of black pepper, representing 12 varieties, and 25 samples of white pepper, including 9 varieties. These are summarized in Table LVIII.

In Table LIX is given for comparison similar analyses compiled from various sources, of common adulterants of pepper.

[1] *Michigan Dairy and Food Commission, Bull.* **94**; Leach: Food Analysis, 3d Ed., p. 432.

TABLE LVII.—ANALYSES OF GENUINE PEPPER[1]

Determination	Black pepper			White pepper		
	Max., per cent.	Min., per cent.	Av., per cent.	Max., per cent.	Min., per cent.	Av., per cent.
Moisture	12.95	10.63	11.86	14.47	12.72	13.47
Total ash	6.85	3.09	5.10	2.96	1.03	1.77
Ash soluble in water	3.20	1.75	2.60	0.80	0.28	0.47
Ash insoluble in acid	1.63	0.00	0.70	0.20	0.00	0.10
Volatile ether extract	2.20	0.65	1.28	0.95	0.49	0.63
Non-volatile ether extract	10.37	6.86	8.41	7.94	6.26	6.91
Alcohol extract	11.86	8.31	9.44	8.55	7.19	7.66
Copper reducing matters by acid conversion	43.47	28.15	38.28	64.92	56.43	59.17
Starch by diastase	39.66	22.05	33.28	63.60	53.11	56.47
Crude fiber	18.25	10.75	13.62	4.25	0.54	3.14
Albuminoids	13.81	10.50	11.93	11.19	10.44	10.89
Total nitrogen	2.53	2.03	2.25	2.13	1.95	2.04
Total nitrogen in non-volatile ether extract	0.39	0.27	0.33	0.34	0.26	0.30

TABLE LVIII.—ANALYSES OF UNADULTERATED PEPPER

Determination	Black pepper			White pepper		
	Max., per cent.	Min., per cent.	Av., per cent.	Max., per cent.	Min., per cent.	Av., per cent.
Moisture	11.96	8.09	9.54	13.34	8.04	9.87
Total ash	7.00	3.43	4.99	4.28	0.86	1.69
Ash soluble in water	3.32	1.65	2.49	1.16	0.12	0.34
Ash insoluble in acid	1.80	0.05	0.58	0.86	0.05	0.19
Starch by diastase	41.75	25.09	36.69	63.55	48.88	54.37
Volatile ether extract	2.10	0.85	1.30	1.66	0.78	1.17
Non-volatile ether extract	10.44	6.60	7.67	7.26	5.65	6.46
Crude fiber	18.89	10.05	11.12	7.65	0.10	4.17
Total nitrogen	2.38	1.86	2.11	2.14	1.85	1.97
Nitrogen in non-volatile ether extract	0.45	0.25	0.31	0.34	0.24	0.30
Albuminoids	13.12	9.25	11.20	11.56	9.69	10.44

[1] Winton, Ogden and Mitchell: *Ann. Rept. Conn. Exp. Sta.*, 1898, 198.

TABLE LIX.—ANALYSES OF PEPPER ADULTERANTS

	Cocoa-nut shells	Spruce saw-dust	Oak saw-dust	Pepper shells	Long pepper	Lin-seed meal	Olive stones	Cocoa shells	Buck-wheat hulls	Ex-hausted cubebs	Ex-hausted allspice
Moisture (per cent.)	7.36	8.77	5.73	11.0– 7.0	10.1– 8.4	8.71	9.50	10.44	7.63	5.60	7.69
Total ash (per cent.)	0.54	0.23	1.22	28.8– 7.8	14.4– 6.1	5.72	0.88	8.40	1.84	10.38	4.50
Ash soluble in water (per cent.)	0.50	0.16	0.32	4.7– 1.5	4.4– 1.7	1.74	0.24	4.66	1.24	6.32	2.59
Ash insoluble in acid (per cent.)	0.00	0.00	0.20	22.9– 0.8	5.9– 0.45	0.55	0.44	0.83	0.00	0.77	0.00
Volatile ether extract (per cent.)	0.00	0.07	0.07	1.1– 0.9	1.0– 0.8	0.04	0.06	1.00	0.07	1.32	0.42
Non-volatile ether extract (per c't.)	0.25	0.77	0.84	4.7– 1.5	7.5– 5.7	6.58	0.24	2.99	0.38	8.58	6.07
Copper-reducing matter by acid conversion (per cent.)	20.88	15.48	17.10	.....	.....	21.15	.....	8.68	20.51	.....	.....
Starch by diastase (per cent.)	0.23	1.13	1.68	11.7– 9.3	45.8–28.4	14.06	1.73	3.15	1.46	8.55	7.42
Crude fiber (per cent.)	56.19	64.03	48.79	22.2–21.0	10.0– 7.2	8.30	57.46	14.12	43.76	27.64	22.89
Total nitrogen (per cent.)	1.13	0.56	1.63	1.8– 1.7	2.1– 2.0	5.09	0.17	2.59	0.17	1.80	1.03

Forms of Adulteration.—Partly because of the extent to which pepper is used, it being the spice most generally added to foods, and partly because its appearance to the eye is not uniform but that of a mixture of particles of different colors, the adulterants of pepper are perhaps more varied than with any other spice.

The kinds of refuse and inert material which are added to pepper have been mentioned already and a fairly complete list of substances which have been reported at various times is given on page 340. The most common of these are probably buckwheat middlings and ground olive stones, in addition to such substances as pepper shells and long pepper, which may be regarded as characteristic adulterants of pepper.

An inferior or low-grade pepper, such as classes C and D of Acheen pepper, containing a large proportion of light berries and empty shells, may be substituted in part for the high-grade Penang or Singapore spice, especially when sold under a definite trade name. To conceal the addition of diluents such as cereals or olive stones, which would detract from the flavor of the mixture, small quantities of pungent spices as cayenne or mustard hulls may be added, and the color may be helped out by such substances as turmeric, charcoal or even coal-tar dyes.

Methods of Analysis

The methods for determining the ash data, the ether extract, starch and crude fiber have already been described or referred to under the general methods for examination of spices, pages 332 to 335.

Nitrogen.—Pepper is one of the food substances in which the Kjeldahl or Gunning method for nitrogen as ordinarily used, does not give correct values. This is due to the piperin, which was shown a number of years ago by Arnold and Wedemeyer[1] to be incompletely decomposed. The modification described on page 26, however, will give perfectly satisfactory results, using about a gram of the sample and carrying out the test exactly as described.

Note.—The determination of nitrogen made as described will

[1] *Z. anal. Chem.*, **1892**, 525.

include both that present in the form of piperin or other alkaloid, and that present in protein form. The latter may be determined, if desired, by subtracting from the total nitrogen the amount of alkaloidal nitrogen determined separately as described below under Piperin. The results in the line marked "Albuminoids" in Table LVII were obtained in this way, multiplying the difference in nitrogen content by 6.25.

Piperin.[1]—Extract 10 grams of ground pepper for 4 hours with anhydrous ethyl ether. Evaporate the ether and determine the nitrogen in the residue by the method on page 26. One cubic centimeter of tenth-normal acid = 0.0285 gram of piperin.

Microscopical Examination.—A synopsis of the microscopical characteristics of pepper and of its chief adulterants will be found in the chapter on the Microscopical Examination of Foods, page 50. The principal adulterants shown by the microscope are excess of pepper shells, nutshells (cocoanut, walnut, almond, etc.), long pepper, olive stones, mustard hulls, cayenne, buckwheat and other cereals, peas and other leguminous seeds, allspice, turmeric, sawdust—in fact any waste material that can be easily reduced to a powder.

Characteristics of Pepper.—The larger works on the microscopy of foods, as Winton,[2] Schimper,[3] or Tschirch and Oesterle[4] illustrate in great detail the structural elements to be found in pepper. Fig. 53, from Winton, shows a great number of elements which may be found in the powdered sample. Many of these tissues, however, are of little analytical importance since they occur but seldom or are so easily disintegrated as to be recognized only by careful study. Those of greatest importance are the masses (*am*) of the minute starch grains, the stone cells (*ast*), the cup-shaped "beaker cells" (*ist*) and the occasional bits of dark brown polygonal cells of the epicarp (*ep*). Crystals of piperin may also show under the microscope (Fig. 103).

White pepper shows hardly anything but the starch, both in masses and as individual grains.

[1] Hartel and Will: *Z. Nahr. Genussm.*, **1917**, 573.

[2] Microscopy of Vegetable Foods.

[3] Mikroskopischen Untersuchungen eer Nahrungs. -und Genussmittel.

[4] Anatomischer Atlas der Pharmakognosie.

Photo-micrographs of some of these tissues are shown in Figs. 101 and 102, page 501.

Adulterants.—Practically all of the list of adulterants given above will be found mentioned either under pepper or among the other spices described on pages 46 to 51, the essential microscopic structures best suited for their identification being noted in each case. It will bear repeating here that the only successful way for the beginner to identify the adulterants is to proceed systematically, one step at a time, as stated on page 46, and to examine known samples of pepper and of the adulterants until he becomes familiar with the structures described. A working

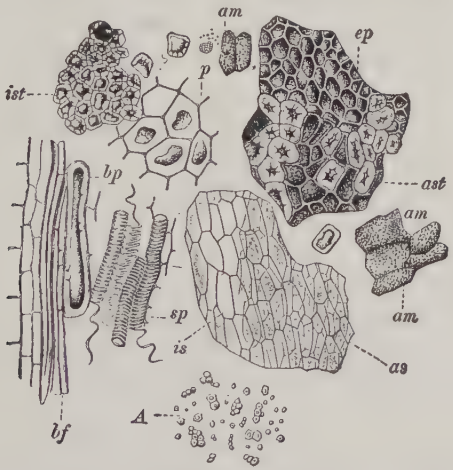


FIG. 53.—Black Pepper. Elements of powder. *ep* epicarp; *ast* hypodermal stone cells; *sp* vessels; *est* endocarp; *am* starch masses. × 120. *A* starch grains, × 450. (MOELLER.)

knowledge of the microscopy of pepper is not difficult to acquire and is of great value in supplementing the chemical analysis.

Color Tests.—Various color tests have been proposed to show adulterants in pepper, especially for ground olive stones and long pepper. A discussion of some of the best of these may be found in Leach[1] and Parry.[2] In the writer's experience, however, they are less delicate and less convenient than the microscopical examination.

[1] *Loc. cit.*

[2] Food and Drugs, Vol. I, p. 206.

Interpretation of Results

The Federal standards[1] for pepper are:

Black pepper is the dried immature berry of *Piper nigrum* L. and contains not less than 6 per cent. of non-volatile ether extract, not less than 25 per cent. of starch, not more than 7 per cent. of total ash, not more than 2 per cent. of ash insoluble in hydrochloric acid, and not more than 15 per cent. of crude fiber. One hundred parts of the non-volatile ether extract contain not less than 3.25 parts of nitrogen.

Ground black pepper is the product made by grinding the entire berry and contains the several parts of the berry in their normal proportions.

White pepper is the dried mature berry of *Piper nigrum* L. from which the outer coating or the outer and inner coatings have been removed and contains not less than 6 per cent. of non-volatile ether extract, not less than 50 per cent. of starch, not more than 4 per cent. of total ash, not more than 0.5 per cent. of ash insoluble in hydrochloric acid, and not more than 5 per cent. of crude fiber. One hundred parts of the non-volatile ether extract contain not less than 4 parts of nitrogen.

By comparison with the tables of analyses of genuine pepper previously given it will be seen that these standards are reasonably liberal and there should be no difficulty in marketing a pepper which will conform to them.

It is seldom necessary in making a chemical analysis of the sample to complete all the tests given in the tables. The requirements laid down in the standards cover the ground quite fully and for many purposes the determinations of non-volatile ether extract, of its nitrogen content, and of crude fiber furnish sufficient information. When black pepper is adulterated with such materials as buckwheat hulls, charred cocoanut hulls, or other substances low in ether extract, the amount of non-volatile ether extract will be diminished without sensibly decreasing the proportion of nitrogen in 100 parts of the extract. When, on the other hand, linseed meal or other oily material has been added, the percentage of non-volatile ether extract may remain about the same as in genuine pepper, but the parts of ni-

[1] *U. S. Dept. of Agr., Office of the Secretary, Circular* **19.**

trogen in 100 of the extract will be decreased. The proportion of nitrogen in the non-volatile ether extract of pure pepper is remarkably constant, varying ordinarily between 3.75 and 4.10 parts per 100.

The starch by diastase and copper-reducing matters by direct acid hydrolysis are both highest in the best grades of black pepper and lowest in the cheapest grades, while the reverse is true of crude fiber, total ash and ash insoluble in acid. The difference between starch by acid and starch by diastase is not nearly so great in pure pepper, especially in white pepper, as in many of the common adulterants. (See Table LIX.) The term starch, as used in the standards, has reference to the diastase method of determination, and inspection of Table LIX will show how easily the presence of some of the adulterants might be overlooked if the simpler method of direct hydrolysis by acid were relied upon entirely. The figures given in the table indicate very clearly the importance of the crude fiber determination in showing adulteration, either with shells or hard vegetable substances.

In the case of white pepper, determinations of ether extract and of nitrogen in the ether extract will again be found the best methods for detecting starchy adulterants. Pure white pepper should contain from 4.0 to 4.5 parts of nitrogen in 100 of ether extract. Such adulterants as olive stones, nutshells and sawdust are indicated chemically by the low content of starch and high values for crude fiber.

For showing the actual character of the adulteration, the microscopical examination is, of course, of paramount importance.

TABLE LX.—ANALYSES OF ADULTERATED PEPPER

	Total ash per cent.	Ash insol. in acid per cent.	Ether extract		Starch per cent.	Crude fiber per cent.	Microscopical
			Vol. per cent.	Non-vol. per cent.			
Black pepper....	3.45	0.35	0.3	9.1	32.9	19.4	Nutshells, cayenne, turmeric.
Black pepper....	6.2	0.85	0.12	6.42	46.4	14.4	Wheat, corn, buckwheat, mustard hulls.
Black pepper....	5.66	2.61	0.24	1.63	.....	47.9	Olive stones and cayenne.
Black pepper....	9.65	2.26	0.11	8.20	31.16	16.2	Excess of shells.
White pepper...	2.25	.....	.....	6.59	.....	3.2	Wheat product.

In Table LX are given a few typical analyses of adulterated pepper.

Cassia and Cinnamon

Although cassia and cinnamon are, as a matter of fact, obtained from different localities and from different species of plant, the terms have been employed so loosely that in common usage they have come to mean the same product. This interchangeability of the names is sanctioned to a certain degree by the standards of the Association of Official Agricultural Chemists,[1] in which *Cinnamon* is defined as "the dried bark of any species of the genus *Cinnamomum* from which the outer layer may or may not have been removed." *Cassia* is stated to be "the dried bark of various species of *Cinnamomum*, other than *Cinnamomum Zeylanicum*, from which the outer layers may or may not have been removed."

A study of the commercial product and its adulterations, however, would hardly be complete without pointing out what differences exist between the two spices.

The product ordinarily sold in the ground condition is undoubtedly cassia. Of this there are three principal varieties, Saigon, Canton or China, and Batavia cassia, consisting of the bark from various species of tree belonging to the laurel family.

Canton cassia is the poorest and cheapest grade, its average wholesale price being given as about half that of Batavia, the next higher, and only one-fifth that of Saigon, the most pungent and expensive of the cassias. The commercial bark, in the case of either variety, may come in large or small pieces, packed in bundles of several pounds weight, or in tightly rolled quills similar to those of true cinnamon but coarser. The latter form is more characteristic of Batavia cassia.

True cinnamon is the bark from the young branches of *Cinnamomum Zeylanicum*, a small tree cultivated mainly in Ceylon. The bark is carefully removed from the branches, scraped, dried and the thin pieces curled one within another into quill-like rolls. These are of a light buff or brown color, streaked with lighter colored wavy lines of bast-fiber bundles, and are quite readily distinguished from the whole cassia.

[1] *U. S. Dept. of Agr., Office of the Secretary, Circular* **19.**

Another portion of the cassia tree, which is used as a spice, is the dried flower buds, known in commerce as *cassia buds*. They are occasionally used in powdered form as a spice directly but more commonly mixed with ground cassia bark. The buds themselves resemble cloves but are smaller and have the odor and flavor of cinnamon.

Composition.—Cassia and cinnamon contain a small quantity, 1–1.5 per cent., of essential oil, to which is mainly due their characteristic flavor. The oil is of a golden color when fresh, with an aromatic odor and a pungent taste, being powerful enough to blister the tongue. The important constituent of the oil is *cinnamic aldehyde*, $C_6H_5-CH = CH-CHO$, of which 60 to 70 per cent. is present.

Besides the oil the bark contains a considerable proportion of starch, some gum, resin, pigment and a small amount of tannin, to the latter of which it doubless owes its somewhat astringent taste.

Analyses of Authentic Samples.—The most extended series of analyses of genuine cassia and cinnamon are those made by Winton, Ogden and Mitchell[1] in their study of the composition of pure spices. These are summarized in the following table:

TABLE LXI.—ANALYSES OF GENUINE CINNAMON AND CASSIA

Determination	Ceylon cinnamon			Cassia bark			Cassia buds
	Max., per cent.	Min., per cent.	Av., per cent.	Max., per cent.	Min., per cent.	Av., per cent.	Av., per cent.
Moisture	10.48	7.79	8.63	11.91	6.53	9.24	7.93
Total ash	5.99	4.16	4.82	6.20	3.01	4.73	4.64
Ash soluble in water	2.71	1.40	1.87	2.52	0.71	1.68	2.88
Ash insoluble in acid	0.58	0.02	0.13	2.42	0.02	0.56	0.27
Volatile ether extract	1.62	0.72	1.39	5.15	0.93	2.61	3.88
Non-volatile ether extract	1.68	1.35	1.44	4.13	1.32	2.12	5.96
"Starch" by acid	22.00	16.65	19.30	32.04	16.65	23.32	10.71
Crude fiber	38.48	34.38	36.20	28.80	17.03	22.96	13.35

The samples reported in the above table were above the average grade and hardly show the variations which may be met in

[1] *Ann. Rept. Conn. Agr. Expt. Sta.*, **1898**, p. 204.

the commercial powdered spice. Owing to varying conditions in the spice market, which may affect the quantity of a standard grade available and its price, the spice grinder is frequently obliged to use small quantities of broken or low-grade spice, blending them in such a way as to get a product which shall conform to the legal requirements and at the same time possess the flavor demanded by his customers.

Table LXII[1] shows the variation in composition that may be met in cassias of this kind.

TABLE LXII.—ANALYSES OF COMMERCIAL CASSIAS

Variety	Total ash per cent.	Water-soluble ash per cent.	Ash insoluble in acid per cent.	Volatile ether extract per cent.	Non-volatile ether extract per cent.	Crude fiber per cent.
Seychelle bark (*a*)	4.08	2.54	0.29	0.66	1.87	49.49
Seychelle bark (*b*)	5.49	2.73	0.07	0.70	1.99	44.66
Ordinary broken China	3.96	0.91	1.24	0.90	2.91	24.84
No. 1 broken Saigon	3.77	1.25	0.05	3.39	4.13	25.29
Extra No. 1 Batavia	2.92	0.71	0.09	2.45	2.95	13.33
Pakhoi rolls	2.62	0.82	0.33	1.16	2.58	21.07
Coarse Corintjie	3.14	1.09	0.48	2.23	3.52	28.16
No. 1 Corintjie	5.97	2.08	0.13	1.33	4.45	19.04
China rolls	2.85	0.64	0.15	1.64	3.32	24.73
Good short Batavia	4.10	1.67	0.19	2.49	4.10	14.08
Kwangsi rolls 3d	3.39	1.45	0.21	2.71	4.45	18.61

METHODS OF ANALYSIS

The usual determinations of total and soluble ash, ether extract, starch and crude fiber are made as described on pages 333 to 335. It should be noted in the determination of starch that since some samples of cassia form with water or dilute alcohol a glutinous mass which clogs the filter, it is best with this spice to omit washing previous to the acid conversion. The determination of calcium oxalate has been recommended as of value in distinguishing between cassia and cinnamon. (See page 348.) It may be carried out as follows:

Calcium Oxalate.—Digest 5 grams of the powdered sample with an excess of dilute hydrochloric acid and filter. Evaporate

[1] Sindall: *J. Ind. Eng. Chem.*, **1912**, 590.

the filtrate to small volume, filter again if necessary, nearly neutralize with ammonia, heat to boiling and add ammonia in slight excess. Acidify with acetic acid and keep at the boiling point for some time. Filter, wash and ignite strongly to CaO.

Microscopical Examination.—A summary of the microscopical characteristics of cassia and of its common adulterants is given in Chapter II, page 47. Fig. 54 shows the elements of powdered cassia as drawn by Moeller. Of these the most important are the spindle-shaped bast fibers (*bf*) with their narrow lumen or central canal (also Fig. 83, page 499); stone cells (*st* and *stp*);

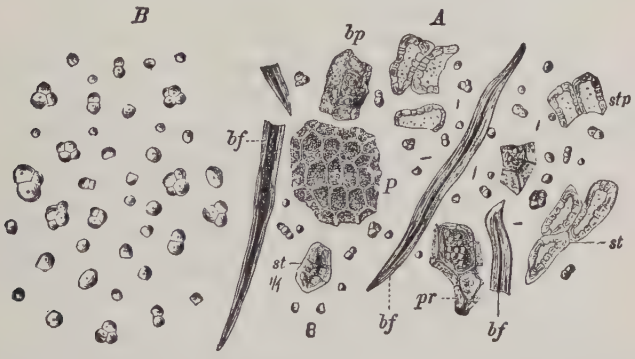


FIG. 54.—China Cassia. *A* elements of the powdered bark: *bf* bast fibers; *st* pericycle stone cells; *stp* cortex stone cells; *P* sclerenchymatized cork. × 120. *B* starch grains, × 450. (MOELLER.)

starch grains (*B*) either singly or in aggregates of two or three; and possibly cork cells (*P*), depending on whether the bark has been scraped or not, may be present. The harder tissues can be studied best after clearing with alkali or chloral hydrate.

Cassia buds do not differ greatly from cassia bark but can be distinguished best by the presence of the trichomes or short, crooked hairs shown at *a* in Fig. 86, page 499.

INTERPRETATION OF RESULTS

The only standard which has been formulated for ground cassia and cinnamon in Circular 19[1] is that it is a "powder consisting of cinnamon, cassia or cassia buds, or a mixture of these spices,

[1] Standards of Purity for Food Products, U. S. Dept. Agr., Office of the Secretary.

and contains not more than 6 per cent. of total ash and not more than 2 per cent. of sand."

The addition of such adulterants as ground bark or sawdust or of cereals would be indicated chemically by the high crude fiber in the first case and the decreased fiber and ether extract in the latter, if these adulterants were used singly. It will readily be seen, however, that the employment of mixtures of these substances, especially when other spices, as allspice or cayenne are judiciously added, would be extremely difficult to detect by chemical methods. They would, on the other hand, be readily apparent in the microscopical examination.

Cassia buds have a higher percentage of non-volatile ether extract, and a lower percentage of crude fiber, than the bark, but otherwise are not essentially different in composition.

Distinction between Cassia and Cinnamon.—Cassia and cinnamon are so closely allied that to distinguish between them is difficult when they are in the form of powder. Cinnamon is lighter in color than cassia, the starch grains are only about half as large, and the bast fibers are somewhat narrower and more numerous. These differences, although noticeable by direct comparison with typical specimens, are, however, rather slight on which to base the actual identification of commercial samples, especially when mixtures may be expected also.

The crude fiber of cinnamon is higher than that of cassia, averaging 36.20 per cent., with a minimum of 34.38 per cent. as against an average of 22.96 per cent. and a maximum of 28.80 per cent. for cassia (see Table LXI); but this difference would be less noticeable with low-grade or only partially cleaned samples. A better distinction has been pointed out by Hendrick[1] in the content of calcium oxalate, which was found to vary from 0.05 to 1.34 per cent. in cassia and from 2.50 to 3.81 per cent. in Ceylon cinnamon. Wild cinnamon contains even more calcium oxalate, two samples showing 6.62 and 6.99 per cent.

In view of the definition of cassia and cinnamon quoted on page 344, however, the distinction possesses more scientific interest than legal value, unless the samples were definitely labeled Ceylon or true cinnamon.

[1] *Analyst*, **1907**, 14.

Cloves

Cloves are the unexpanded flower buds of *Eugenia caryophyllata* Thbg., or *Caryophyllus aromaticus* L., an evergreen tree of the myrrh family, the name of the spice coming from the French word "clou," meaning "a nail," which it somewhat resembles.

The clove tree is indigenous to the Molucca or so-called "Spice Islands," but is now cultivated in Guiana, Ceylon, India, Zanzibar, and the East and West Indies. The chief varieties of commerce, named from their place of growth and graded in value in the order named, are Penang, Amboyna and Zanzibar. The flowers are of a delicate pink color and grow in clusters of from nine to fifteen. During growth the green buds change to red and are then ready for harvesting. If they are allowed to remain on the tree several weeks longer they swell, forming an oblong berry containing several seeds. The fruit is then ripe and is known as the "mother clove" or clove fruit. It will have lost its pungency at this point, however, so that it is only the unripe buds that are gathered. At the proper time the buds are hand-picked or beaten from the tree with slender reeds, cloths being spread beneath to catch them. The only further treatment they receive is drying in the sun, which changes them to a rich brown color, or occasionally smoking over a fire which imparts to the product a much darker tint.

The clove has a long cylindrical calyx, dividing above into four pointed spreading sepals, which surround four petals or leaves that are the unexpanded flowers. These are rolled into a globular bud at the head of the clove. The parts may be seen by soaking the clove in water, when the leaves will soften and unroll. The lower end of the calyx, of a deep, rich brown color, is solid, with a dull wrinkled surface and dense fleshy texture. It abounds in essential oil which exudes on simple pressure with the finger nail.

General Composition.—Cloves are somewhat different from the spices that have just been discussed with respect to the very large proportion of volatile oil that is present, a larger percentage of essential oil, in fact, than is found in any other natural food product. In addition there are found also resin, tannin

and a small amount of albuminoids. No starch is present. The average composition might be summarized as below:

	Per cent.
Water	8.5
Ash	6.0
Essential oil	16.5
Fixed oil and resin	7.5
Tannin	18.0
Albuminoids	6.0
Fiber and cellulose	8.0

The most interesting constituent is the volatile oil, which may be readily obtained by distillation with steam and has a pungent taste and the characteristic odor of cloves. It consists mainly of *eugenol*, $C_{10}H_{12}O_2$, the amount present being from 80 to 95 per cent. Small quantities of eugenol esters and of the sesquiterpene, *caryophyllene*, are also found in the oil.

Authentic Analyses.—Analyses of genuine cloves, including the several varieties, have been reported by Richardson,[1] McGill[2] and Winton, Ogden and Mitchell.[3] Of these the analyses by the latter authorities, made by the same methods that were employed for the spices previously discussed, are tabulated below:

TABLE LXIII.—ANALYSES OF GENUINE CLOVES

	Cloves			Clove stems
	Maximum per cent.	Minimum per cent.	Average per cent.	Average per cent.
Moisture	8.26	7.03	7.81	8.74
Total ash	6.22	5.28	5.92	7.99
Water-soluble ash	3.75	3.25	3.58	4.26
Ash insoluble in acid	0.13	0.00	0.06	0.60
Volatile ether extract	20.53	17.82	19.18	5.00
Non-volatile ether extract	6.67	6.24	6.49	3.83
"Starch" by acid	9.63	8.19	8.99	14.13
Starch by diastase	3.15	2.08	2.74	2.17
Crude fiber	9.02	7.06	8.10	18.71
Total nitrogen	1.13	0.94	0.99	0.94
"Oxygen equivalent"	2.63	2.08	2.33	2.40

[1] *Bur. of Chem., Bull.* **13,** Part II, p. 225.
[2] *Canada Int. Rev. Dept., Bull.* **73,** p. 6.
[3] *Ann. Rept. Conn. Agr. Expt. Sta.,* **1898,** p. 206.

McGill's analyses of genuine whole cloves, which are somewhat similar but include a greater number of samples, are summarized in the following table:

TABLE LXIV.—ANALYSES OF DIFFERENT VARIETIES OF CLOVES

Variety	Moisture per cent.	Volatile oil per cent.	Petroleum ether extract per cent.	Fixed oil per cent.
Penang:				
Maximum	7.4	24.3	28.2	12.0
Minimum	5.0	20.7	24.4	9.5
Average	6.2	22.4	27.0	10.8
Amboyna:				
Maximum	6.7	25.9	29.2	10.0
Minimum	5.5	23.5	26.5	8.2
Average	6.1	24.6	27.5	9.0
Zanzibar:				
Maximum	6.7	23.6	28.1	10.7
Minimum	4.1	18.6	21.3	8.0
Average	5.7	21.7	25.5	9.6

In making the analyses reported in this table the *moisture* was determined by drying the ground sample over sulphuric acid *in vacuo* for 24 hours; the total *volatile matter* by drying the ground sample at 98°C. for 18 hours; and the *petroleum ether extract* by the usual methods in a continuous extraction apparatus. The *volatile oil* was obtained by subtracting the moisture from the total volatile matter.

METHODS OF ANALYSIS

Moisture, ash, volatile and non-volatile ether extract, starch and crude fiber are determined by the methods already described on pages 333 to 335.

With a product containing so much volatile oil the moisture is best determined byMcGill's method[1] in which a weighed portion (2 grams) of the ground sample is kept over sulphuric acid at about 60 mm. pressure for 24 hours, by which the whole of the aqueous vapor is absorbed by the sulphuric acid while only traces of the essential oil are removed.

[1] *Loc. cit.*

Tannin.—Since the quantity of tannin present in cloves is fairly constant and considerably higher than in the common adulterants or other spices, the determination is of some value for this spice. The usual method of oxidation by potassium permanganate may be used, having previously removed the easily oxidized oil by extraction with ether.

Reagents.—(*a*) *Tenth-normal potassium permanganate solution,* which may be standardized against ferrous ammonium sulphate or pure oxalic acid as described in any standard book on quantitative analysis.

(*b*) *Indigo solution,* made by dissolving 0.6 gram of the best sodium sulphindigotate[1] in 50 cc. of water, with the aid of heat, cooling, adding 5 cc. of concentrated sulphuric acid, making up to 100 cc. and filtering.

Process.—Extract 2 grams of the sample with anhydrous ethyl ether for 20 hours, or use the residue from the determination of "ether extract" if desired. In either case boil the residue for 2 hours with 300 cc. of water, cool, make up to 500 cc. and filter.

Measure 25 cc. of the filtrate into a liter flask or into a 12-in. porcelain evaporating dish. Add 20 cc. (measured) of the indigo solution and 750 cc. of distilled water. Titrate the solution with the permanganate, stirring or shaking thoroughly, until the blue color of the solution begins to change to green, then add the permanganate more slowly until the color changes to greenish yellow. Allow the liquid to stand for a moment or two, and then add the permanganate a drop at a time with thorough mixing, until a bright golden yellow color is obtained.

In the same way note the number of cubic centimeters of permanganate required to oxidize the 20 cc. of indigo solution alone. This amount, subtracted from the previous reading, leaves the quantity of permanganate needed to oxidize the tannin.

The result may be expressed as "oxygen equivalent," "oxygen absorbed" or directly as quercitannic acid. One cubic centimeter of $\frac{N}{10}$ permanganate is equivalent to 0.0008 gram of oxy-

[1] Only the purest indigo salt should be used since otherwise the end-point is indefinite. The indigocarmin prepared by G. Gruebler and Co. of Leipsic and sold by dealers in microscopic staining material, is well adapted for the purpose.

gen, or from Neubauer's value for the reduction-equivalent of oak-bark tannin, to 0.006235 gram of quercitannic acid.

Notes.—The method is based on the fact, first worked out by Lowenthal,[1] that tannin is oxidized in acid solution by permanganate. The oxidation, however, proceeds slowly and the end-point is indefinite. By the addition of a considerable quantity of indigo the oxidation of the tannin is controlled, and the end-point can be recognized by the change in color.

In order to insure a uniform limiting action, the volume of permanganate which is used in a titration should not greatly exceed one and a half times that which is required for the indigo alone, and the titrations should be carried out in a strictly similar manner as regards stirring and rate of addition of the permanganate.

It should be pointed out that as described, the method does not determine the actual amount of tannin, since some other oxidizable substances still remain, although the greater part are removed by the extraction with ether. The more exact determination, however, involving the use of gelatin or hide powder to separate the tannin, would be much more tedious and the results would be no more useful.

The value of the method is indicated by the following figures obtained with cloves and the common adulterants.

Materials	Per cent. of "oxygen absorbed"	Per cent. of "quercitannic acid"
Cloves	2.35	18.5
Clove stems	2.40	18.8
Almond shells	0.40	3.2
Date stones	0.61	4.7
Spruce sawdust	0.30	2.4
Cocoanut shells	0.47	3.7
Linseed meal	1.00	7.8
Cocoa shells	1.26	9.7

Microscopical Examination.—Powdered cloves, under the microscope, differ somewhat from the typical spices that have been considered previously in that practically no characteristic elements can be seen without close study. The general appear-

[1] *Z. anal. Chem.* **1877,** 33.

ance is that of a confused mass of dark-colored cellular tissue. Careful rubbing out under the cover glass between the thumb and finger, as described on page 35, will help in breaking up the masses, and treatment of the slide with chloral hydrate as explained on the same page, will be of value. Even under the best conditions, however, the only special forms that will be readily apparent are the bast fibers, of which cloves contain a few. This lack of distinctly characteristic microscopical elements is in a way an advantage in that it renders the detection of foreign tissues easier.

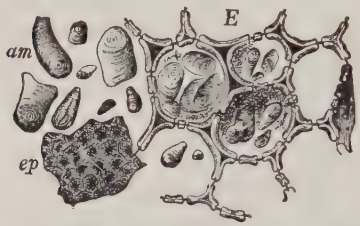


FIG. 55.—Mother Cloves. Elements of seed. *ep* epidermis and *E* ground tissue of cotyledon; *am* starch grains. × 225. (MOELLER.)

The common adulterants which the microscope will show are clove stems, mother cloves, nutshells, fruit stones, allspice, and cereals.

Clove stems are distinguished from cloves by the greater number of bast fibers present, by the numerous stone cells (cloves have practically none) which much resemble those of all-

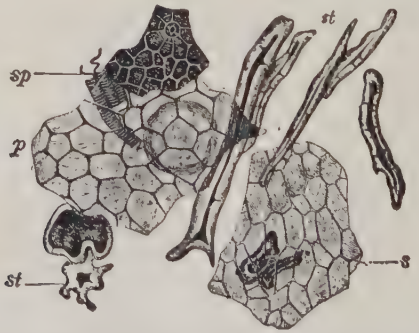


FIG. 56.—Mother Cloves. Tissues of pericarp in surface view. *sp* spiral vessels and epicarp; *p* brown parenchyma with underlying oil cavity; *st* stone cells and fibers. × 120. (MOELLER.)

spice (Fig. 76, page 497), but are not accompanied by the lumps of colored resin; and especially by the characteristic bundles of reticulated and scalariform vessels shown in Figs. 77 and 88, pages 497 and 499.

Mother cloves are characterized by the presence of large starch grains (*am*, Fig. 55), resembling quite closely those of sago, and very peculiar irregular, twisted and knotted stone cells, (*st*, Fig. 56).

Nutshells and *fruit stones*, typical examples of which are cocoanut shells and olive stones, are recognized by their characteristic long and spindle-shaped stone cells as indicated under Allspice, page 46.

Allspice itself, being considerably cheaper than cloves and resembling the latter spice both in appearance and odor, is a favorite adulterant. Its characteristic elements are outlined on page 46, the feature best adapted for showing its presence in cloves being the colored resin. Fig. 89, page 499, is a photograph of cloves adulterated with ground allspice.

Other less characteristic adulterants, as ginger or the cereals, are also to be looked for and can be identified by the description recorded in Chapter II.

Interpretation of Results

The United States standard for cloves defines them as "the dried flower buds of *Caryophyllus Aromaticus*, which contain not more than 5 per cent. of clove stems; not less than 10 per cent. of volatile ether extract; not less than 12 per cent. of quercitannic acid; not more than 8 per cent. of total ash; not more than 0.5 per cent. of ash insoluble in hydrochloric acid; and not more than 10 per cent. of crude fiber.

McGill,[1] as a result of a study of ground cloves sold in Canada and comprising 140 samples, suggests standards similar to the above, except that in place of the volatile ether extract, the minimum for volatile oil (see page 351) is fixed at 14 per cent.

Since the standards, however, are designed to include all samples of reasonable purity to which no additions of foreign material have been made, it will be found that the larger proportion of genuine samples will fall well within these limits. McGill, for example, points out that the ash of pure cloves will, in general, fall within 6 per cent. and the acid-insoluble ash within 0.3 per cent. This is equivalent to saying that the

[1] *Canada Inland Revenue, Bull.* **252, p. 5.**

majority of genuine samples, and certainly if of high grade, will not approach the extreme limits of the standards.

The most valuable chemical determination is undoubtedly the volatile oil or volatile ether extract, and the result of this test, taken together with the ash data and possibly the tannin, will usually serve to indicate the extent to which any non-starchy adulterant has been added. Since cloves contain no starch, testing with iodine solution will show the presence of added starchy material and the test may be readily confirmed by the microscopical examination.

Clove stems, a common adulterant, have only one-fourth as much volatile oil, half as much non-volatile ether extract, and more than twice as much crude fiber as cloves. The presence of stems as an adulterant also notably increases the ash and the acid-insoluble ash.

The presence of exhausted cloves is most readily shown by the decreased percentage of essential oil or volatile ether extract. The exhausted cloves, if whole, can be recognized by their shriveled and striated appearance, as well as the very dark, almost black color. According to Parry[1] the use of exhausted cloves is greater than might be imagined, one essential oil distillery, to the author's knowledge, having an annual output of 40 tons of such material.

Quite similar results to those obtained by the admixture of exhausted cloves, that is, a decrease in the volatile ether extract, soluble ash and tannin, would be shown by a mixture of allspice with cloves, a form of adulteration which is not at all uncommon. This is shown clearly in the average figures for allspice given below. The characteristic tissues of allspice, however, render its detection by means of the microscope comparatively easy.

COMPOSITION OF ALLSPICE

	Per cent.
Total ash	4.50
Ash soluble in water	2.50
Volatile ether extract	4.00
Non-volatile ether extract	5.75
Starch by diastase	3.00
Crude fiber	22.00
Oxygen equivalent	1.20
Quercitannic acid	9.7

[1] Food and Drugs, Vol. I, p. 222.

Artificial cloves made from dough, powdered bark and clove powder, and from soft wood stained a dark color and soaked in a solution of oil of cloves, have been reported, but while interesting are too rare to be of any importance. Apart from the detection of exhausted cloves, the microscope must be regarded as the chief reliance of the analyst in the examination of cloves.

Mustard

Source.—The mustard of commerce is the seed, whole or powdered, of several cruciferous plants of the genus *Brassica*. It is an annual herb, from 3 to 6 ft. high, with a yellowish flower and a small pod containing round seeds, and is a common plant in both Europe and America.

The plant is cultivated for spice largely in Bohemia, Holland and Italy, certain parts of England, and in California and Kentucky of the United States.

Although numerous varieties of the mustard plant are known, only two are of commercial importance, black or brown mustard (*Brassica nigra*), of which the best comes from Italy through the Austrian port of Trieste, and white or yellow mustard (*Brassica alba*), the best grades of which come from England and Holland.

Manufacture.—Mustard seed contains so much oil that it cannot be ground between stones in the ordinary manner of grinding spices. It is crushed by passing between rollers, then pressed by hydraulic presses to extract the oil, after which the dried residue or "mustard cake" is put into pots and pounded by powerful stamps until the material is reduced to the desired powder. It is then sifted on sieves of silk cloth to separate the coarse hulls from the fine flour. The yield of flour varies from 30 to 60 per cent. of the weight of the seed. The residue on the sieves is frequently used in the manufacture of "prepared" or "French" mustard, a paste composed ordinarily of ground mustard seed, salt, spices and vinegar.

Composition.—The general composition of mustard seed is shown in the following analysis of black and white mustards by Piesse and Stansell:[1]

[1] *Analyst*, **1880**, 163.

	White mustard per cent.	Black mustard per cent.
Water	8.00	8.52
Non-volatile oil	27.51	25.54
Cellulose	8.87	9.01
Albuminoids	28.06	26.50
Myrosin and albumin	4.58	6.46
Soluble matter	26.29	24.22
Volatile oil	0.08	0.47
Ash	4.70	4.98

Both varieties of mustard are thus seen to contain considerable quantities of non-volatile oil, albuminoids and mucilaginous material, but no starch. This fixed oil is entirely lacking in pungency, being a bland, tasteless oil somewhat resembling olive oil, and is to some extent used as an adulterant of the latter. (See page 178.)

The interesting constituent of the mustard is the volatile oil. This is not present in the mustard as such but is developed by the addition of cold water. The actual substance present is *sinigrin* or potassium myronate ($KC_{10}H_{16}NS_2O_9$), a glucoside which by hydrolysis, through the agency of the enzymes also present, splits into glucose, potassium acid sulphate and allyl isothiocyanate (C_3H_5CNS). The latter is the pungent volatile mustard oil. The reaction may be expressed:

$$KC_{10}H_{16}NS_2O_9 + H_2O \rightarrow C_6H_{12}O_6 + HKSO_4 + C_3H_5CNS$$

White mustard contains a somewhat similar glucoside, *sinalbin* ($C_{30}H_{42}N_2S_2O_{15}$). This splits in an analogous manner to the sinigrin, forming *sinalbin mustard oil* (C_7H_7ONCS), which in pungency much resembles the volatile oil from black mustard.

Analyses of Authentic Samples.—The composition of mustard seed, flour and hulls, as shown by the usual methods of analysis for spices, is summarized in Table LXV.[1]

These mustard "flours" were prepared by the commercial methods, from seeds separated as thoroughly as possible from the hulls, and having the usual proportion of fixed oil removed by pressure.

[1] Leach: *J. Am. Chem. Soc.*, **1904**, 1203.

TABLE LXV.—ANALYSES OF GENUINE MUSTARD

	Whole Seeds.			Flour.			Hulls.		
	Max. per cent.	Min. per cent.	Av. per cent.	Max. per cent.	Min. per cent.	Av. per cent.	Max. per cent.	Min. per cent.	Av. per cent.
Moisture	6.82	5.88	6.37	9.50	5.09	6.96	9.12	5.36	7.48
Total ash	4.83	3.84	4.25	5.58	4.66	5.03	5.03	4.43	4.65
Water-soluble ash	0.73	0.45	0.56	0.27	0.09	0.19	2.33	0.95	1.67
Ash insol. in HCl	0.56	0.16	0.32	0.50	0.08	0.27	0.22	0.04	0.12
Volatile ether extract	0.0	0.0	.....	0.0	0.0	.....	0.0	0.0	.....
Non-volatile ether extract	37.81	27.19	31.22	25.95	12.65	18.59	13.81	6.17	8.56
Total nitrogen	5.09	3.96	4.41	7.44	6.21	6.78	4.04	2.90	3.45
Crude fiber	6.53	4.21	5.04	3.28	1.87	2.42	18.95	10.90	15.20
"Starch" by acid hydrolysis	10.06	6.94	8.62	11.89	4.87	6.85	20.40	9.90	16.96
"Starch" by diastase	1.82	0.92	1.48	0.71	0.00	0.28	6.11	1.20	3.62
Alcohol extract	17.75	13.70	15.50	25.31	19.22	22.30	14.21	8.07	11.07

ANALYTICAL METHODS

Tests for the amount of ash, ether extract, crude fiber, starch, etc., are made by the usual methods applicable to spices and already described.

These methods will ordinarily give all the needed information regarding the purity or quality of a sample of mustard. If, however, it is desired to make an actual determination of the amount of volatile mustard oil, Roeser's method[1] will be found as convenient as any.

Volatile Mustard Oil.—The method depends on the fact that the mustard oil may be distilled into ammonia, forming thiosinamine,

$$C_3H_5CNS + NH_3 \rightarrow CS\begin{matrix}NH.C_3H_5\\NH_2\end{matrix}$$

This latter when treated with silver nitrate precipitates an amount of silver sulphide corresponding to its content of sulphur, from which it may readily be determined. The method is:

Mix 5 grams of the powdered mustard with 60 cc. of water and 10 cc. of alcohol and allow it to stand for 2 hours. Distil about two-thirds of the solution into a flask containing 10 cc.

[1] J. Roeser: *J. Pharm. Chim.*, **1902,** 361; Leach: Food Inspection and Analysis, 3d Ed., p. 457.

of ammonia, mix the distillate with 10 cc. of $\frac{N}{10}$ silver nitrate solution and allow the mixture to stand for 24 hours. Make up to 100 cc., filter and add to 50 cc. of the filtrate 5 cc. of $\frac{N}{10}$ potassium cyanide solution. Titrate the excess of potassium cyanide with the $\frac{N}{10}$ silver nitrate, using 8 drops of a 5 per cent. potassium iodide solution made slightly ammoniacal as an indicator.

Twice the number of cubic centimeters of silver nitrate used up, multiplied by the factor 0.3137, gives the percentage of mustard oil.

It should be noted that the factor given is not the theoretical one corresponding to the formula of allyl isothiocyanate but an arbitrary one in which allowance is made for the incompleteness of the reaction.

Coloring Matter.—The presence of coal-tar dyes may be shown by digesting the sample for several hours with water containing a few drops of ammonia, filtering, acidifying very slightly with hydrochloric or acetic acid and dyeing on wool, as described on page 60. If enough color is present it may be stripped from the wool and identified by the systematic procedure given in Table VII, page 67.

Turmeric may be detected by the microscope or in an alcoholic extract of the mustard by the boric acid test given on page 58.

Microscopical Examination.—The general characteristics of mustard flour, as viewed in the microscope, are summarized on page 49. Since it is common practice to mix the flour of the black and the white mustard, the commercial product will frequently exhibit the characteristics of both varieties. The bulk of the material consists of protein and fat, with occasional fragments of the palisade layer of the hull (Fig. 99, page 501), gray and black in the case of the white mustard and brown and black with black mustard. Groups of the mucilaginous epidermal cells (Fig. 98, page 501,) are also usually present.

Turmeric is readily identified by the brilliant greenish-yellow

color of the "paste-balls," which become reddish brown if a little alkali is drawn under the cover glass.

Since mustard contains no starch, the addition of cereals or similar adulterants can readily be shown by the iodine reaction and the different starches identified microscopically.

A common adulterant is *charlock*, or wild mustard, which is found as a weed in the wheat fields of the Northwest. This is easily recognized by the fact that the palisade cells, which somewhat resemble those of genuine mustard, give a deep red color when treated with chloral hydrate. To a small quantity of the material on a slide add two drops of a solution of 16 grams of chloral hydrate and 1 cc. of strong hydrochloric acid in 10 cc. of water. Heat gently and examine with a low power. The palisade cells of charlock will become a deep blood red.

Interpretation of Results

The Federal standards[1] define "*ground mustard*" as a powder made from mustard seed, with or without the removal of the hulls and a portion of the fixed oil, and containing not more than 2.5 per cent. of starch and not more than 8 per cent. of total ash.

It will be observed in this standard that the hulls need not necessarily be removed in the preparation of the mustard flour. In the common acceptance of mustard flour, however, the presence of any considerable quantity of hulls would be considered an adulteration, and, indeed, reference to the table on page 359 indicates that by limiting the amount of starch which may be present the proportion of hulls is to a certain extent limited also. The other determinations which would be of the greatest value in showing the presence of excessive amounts of hulls are the soluble ash and the crude fiber.

Such adulterants as turmeric, cayenne, charlock or cereals are mentioned under the heading "Microscopical Examination," since this is by far the readiest means of showing their presence. The claim is sometimes made that turmeric is needed to tone down the pungency of the product and add to its keeping

[1] *U. S. Dept. of Agr., Office of the Secretary, Circ.* **19.**

quality.[1] This, however, can be done by using a greater proportion of the yellow seed in the ground mixture.

Mineral adulterants are rarely seen now-a-days, but if present would be readily shown in the ash determination.

The actual relative value of different samples as regards pungency can best be determined from the amount of allyl isothiocyanate produced when mixed with water.

A typical analysis of the "Dakota" wild mustard which is sometimes mixed with or substituted for the lower grade of genuine mustard is given below. This sample was found by microscopical examination to be free from hulls, and the copper-reducing matter determined by diastase is actual starch.

DAKOTA MUSTARD FLOUR

	Per cent.
Total ash	7.80
Soluble ash	0.46
Ash insoluble in acid	0.75
Ether extract	12.23
Total nitrogen	6.84
Volatile oil	3.76
Crude fiber	2.28
Starch by diastase	2.58

Selected References

BROOKS.—Federal Spice Standards.

GIBBS.—The Spices and How to Know Them.

RICHARDSON.—Spices and Condiments. U. S. Dept. of Agr., Bur. of Chem., Bull. 13, Part II.

WINTON, OGDEN AND MITCHELL.—Analyses of Authentic Spices. Ann Rept. Conn.Agr. Expt. Station, 1898, 1899.

[1] Gibbs: The Spices and How to Know Them, p. 167.

CHAPTER IX

CIDER VINEGAR

General.—Cider vinegar may be defined as the product made by the alcoholic and subsequent acetic fermentation of fresh apple juice. Vinegar is made also by the similar fermentation of wine, malted cereals or other saccharine substances yielding *wine*, *malt* or *sugar* vinegar according to the material employed. The acetic fermentation in the case of cider vinegar will take place spontaneously but is much aided by the addition of vinegar containing some of the "mother," a felt-like scum which forms on the surface of cider during its change to vinegar and contains the various microorganisms which bring about the fermentation.

The process may be carried out by allowing the cider to stand for a long time in casks which are open to the air, but on a commercial scale the vinegar is usually made by allowing the alcoholic liquid from a large storage tank to trickle slowly through a column of shavings, rattan or other fibrous material impregnated with old vinegar. A current of air is allowed to pass upward through the "generator" at the same time so that the fermentation is greatly hastened. The process is under exact control and yields a much more uniform product than is possible under the older barrel method.

The fermented product contains acetic acid, traces of alcohol, aldehydes and other volatile bodies, as well as some of the unchanged sugar and the glycerin resulting from the fermentation.

Forms of Adulteration.—The adulteration of cider vinegar may consist in the substitution of a product almost entirely artificial, but imitating the genuine article in appearance and acidity; in the admixture or substitution of vinegar from another source, as glucose or sugar vinegar, but so manipulated as to conform to many of the analytical constants of genuine cider vinegar; finally, in the "improvement" of a low-grade or inferior product by the addition of cheap material so that it shall simulate the standard article. On account of the low price at which vine-

gar must be sold and the small margin of profit to the manufacturer, much ingenuity has been displayed in the manipulation of inferior grades so that they shall conform to the legal standards.

Typical practices consist in the addition of water, as in the reduction of vinegar of high acid content to legal standard, which reduces not only the acid strength, but the amount of other ingredients in the same proportion. This means that other materials, high in solids and reducing sugars, must be added also. For this purpose, boiled cider, cheap cider jelly or unfermented apple juice are commonly used. Dilute acetic acid, derived from the dry distillation of wood and not from the acetic fermentation of alcohol, may be used to bring back the acid strength.

Perhaps more common, however, is the admixture, or even occasional substitution of distilled vinegar, called also spirit or grain vinegar. This is made by submitting to acetic fermentation a dilute alcohol obtained by the fermentation with yeast and subsequent distillation of a mash of corn, malt and rye. This distilled vinegar, colored with caramel, may be substituted entirely for the cider vinegar, but is usually mixed with the latter in varying amount and the analytical constants restored to normal by the addition of apple waste or boiled cider, high in solids and sugar.

Vinegar may be made from the cheaper grades of cane sugar or low-grade molasses which in many respects closely resembles cider vinegar and is much less easily detected than the admixture with distilled vinegar.

Certain waste products of the apple itself are also utilized in the manufacture of spurious cider vinegars, such as "apple waste" from the skins, cores and chops of the dried-apple industry.

"*Second Pressings.*"—In the manufacture of cider vinegar. the "pomace" from which the apple juice has been expressed is sometimes allowed to stand in piles for several days, during which time it becomes much heated and a fermentation ensues accompanied by partial decomposition, so that when the material is subjected again to high pressure a greater yield of material of rather questionable character is secured.

It is quite easy to imitate some of the characteristics of cider vinegar but difficult to alter all the analytical constants so that adulteration shall not be shown.

Methods of Analysis

The sample should be thoroughly mixed before analysis and if turbid should be filtered. The results may be expressed as per cent. by weight or as grams per 100 cc. The latter method is simpler and for general purposes entirely satisfactory.

Specific Gravity.—Determine at $\frac{15°.6}{15°.6}$ as directed under General Methods, page 1.

Solids.—Measure 10 cc. into a weighed flat-bottom platinum dish of 50 mm. diameter, evaporate on the water-bath to a sirup, dry for 2½ hours in a boiling water-oven, cool in a desiccator and weigh.

Note.—This is the official method of the Association of Official Agricultural Chemists. Owing, however, to the persistent retention of acetic acid in the solids, more accurate results are obtained by adding 5 cc. of water to the residue and again evaporating to dryness, making three evaporations in all before cooling and weighing.

Ash.—Use the residue from the previous determination or, better, measure a fresh portion of 25 cc. into a weighed platinum dish and evaporate to dryness on the water-bath. Ignite at a low red heat until thoroughly charred, leach with water, burn the insoluble residue to whiteness, add the water solution, evaporate and heat at low redness, cool in a desiccator and weigh.

Solubility and Alkalinity of Ash.—Determine as directed on page 18. Express the result of the titration as the number of cubic centimeters of tenth-normal acid per 100 cc. of sample.

Alcohol.—To 100 cc. add saturated sodium hydroxide until faintly alkaline, add a piece of paraffin the size of a pea and distil almost 50 cc. Make up to 50 cc. at the temperature of the sample, filter if necessary, and determine the specific gravity with a pyknometer as on page 3. Calculate the per cent. of alcohol by volume from the table on page 420.

Total Acidity.—Dilute 10 cc. with distilled water until the solution is very light colored, add phenolphthalein and titrate with tenth-normal sodium hydroxide. Calculate as acetic acid, $HC_2H_3O_2$.

Fixed Acid.—Evaporate 10 cc. just to dryness in a porcelain

dish on the water-bath, add 5 to 10 cc. of water and again evaporate; repeat until at least five evaporations have been made and no odor of acetic acid can be detected. Add nearly 200 cc. of recently boiled distilled water, and titrate with tenth-normal sodium hydroxide and phenolphthalein. Express the result as malic acid, $H_2C_4H_4O_5$

Volatile Acid.—Calculate the fixed acid as acetic and deduct from the total acid. Express the result as acetic acid.

Polarization.—Add to 50 cc. of the vinegar, in a 50–55-cc. flask, 2 to 5 cc. of basic lead acetate, make up to the mark, shake and allow to stand 30 minutes. Filter and polarize in a 200-mm. tube, correcting for the dilution.

Reducing Sugar before Inversion.—Evaporate 25 cc. to 5 cc. on the water-bath. Add 25 cc. of water and evaporate again to 5 cc. Wash into a 100-cc. graduated flask; make up to the mark, and determine reducing sugar in 50 cc. by the Munson and Walker method, page 237. Express the results as grams of invert sugar in 100 cc. of sample.

Note.—Vinegar contains varying amounts of volatile reducing bodies which should be eliminated by evaporation to get the true amount of sugar.

Reducing Sugar after Inversion.—Proceed as in the preceding paragraph. After the second evaporation, transfer to a 100-cc. flask with 70 cc. of water and invert by the Herzfeld method, page 257. Nearly neutralize with sodium hydroxide, fill to the mark and determine reducing sugar in 50 cc. as above.

Soluble and Insoluble Phosphoric Acid.—For the *soluble phosphoric acid* use the solution left after determining the alkalinity of the soluble ash. Concentrate, if necessary, make up to 50 cc. in a graduated flask and use 25 cc. Add 2 cc. of nitric acid (sp. gr. 1.20) and 10 grams of ammonium nitrate. Add 25 cc. of ammonium molybdate solution,[1] stir

[1] Prepared by dissolving 100 grams of molybdic acid in 144 cc. of ammonium hydroxide (sp. gr. 0.90) and 271 cc. of water; slowly and with constant stirring pour the solution thus obtained into 489 cc. of nitric acid (sp. gr. 1.42), and 1148 cc. of water. Keep the mixture in a warm place for several days or until a portion heated to 65°C. deposits no yellow precipitate of ammonium phospho-molybdate. Decant the solution from the sediment and preserve for use.

thoroughly and place on a water-bath at a temperature of 40°C. to 60°C. When clear, test 5 cc. with warm ammonium molybdate. If no further precipitation occurs, allow the solution to stand for an hour at 40° to 60°C., filter on a Gooch crucible, using an asbestos felt about ¼ in. thick, and wash twice with water, then with 2 per cent. potassium nitrate solution until 10 cc. of the filtrate gives a pink color with 1 drop of tenth-normal sodium hydroxide and phenolphthalein. Transfer the precipitate and the asbestos to the beaker used for the molybdate precipitation, add tenth-normal sodium hydroxide until the precipitate is all dissolved and the solution colorless (25 cc. is ordinarily sufficient); add 1 cc. of phenolphthalein solution and titrate with tenth-normal hydrochloric acid for the excess of alkali. Calculate the result as milligrams of P_2O_5 in 100 cc. of vinegar (1 cc. of tenth-normal alkali = 0.0003088 gram of P_2O_5). A blank should be run at the same time, using the same amount of reagent, in order to correct for any precipitation of the molybdate solution.

For the *insoluble phosphoric acid* exhaust the "insoluble ash" by boiling with several small portions of hot water acidulated with nitric acid, neutralize with ammonia, make up to 50 cc., take 25 cc. and proceed as in the determination of soluble phosphoric acid.

Alcohol Precipitate.—Evaporate 100 cc. of vinegar to about 15 cc., add 200 cc. of 95 per cent. alcohol slowly and with constant stirring, and allow the mixture to stand over night. Filter and wash with 80 per cent. (by volume) alcohol. Wash the precipitate from the filter with a jet of hot water into a platinum dish; evaporate, dry at 100°C. and weigh. Ignite and weigh again. The loss in weight is the alcohol precipitate.

Note.—If the sugar content of the vinegar is high, the evaporation should not be carried beyond 20 cc., otherwise on adding the alcohol, the precipitate is gummy and stringy rather than flocculent.

Pentosans.—To 100 cc. of vinegar add 43 cc. of hydrochloric acid (sp. gr. 1.19) and carry out the distillation and determination as described on page 264.

Formic Acid.[1]—*Method.*—Add 0.4 to 0.5 gram of tartaric

[1] Fincke: *Z. Nahr. Genussm.*, **1911,** 1; **1912,** 88.

acid to 100 cc. of vinegar and distill with steam. Pass the steam, after leaving the vinegar, through a boiling mixture of 15 grams of calcium carbonate and 100 cc. of water and keep this volume constant throughout the process. Collect 1000 cc. of distillate and reduce the volume of the sample to 30–40 cc. during the distillation. Reject the distillate. Filter and wash the calcium carbonate mixture, make the filtrate and washings, which should not exceed 140 cc. in volume, faintly acid with hydrochloric acid, add 10 to 15 cc. of mercuric chloride solution (10 grams of mercuric chloride and 3 grams of sodium chloride in 100 cc.) and heat in a boiling water-bath for 2 hours. Filter on a Gooch crucible, wash with water, alcohol and finally ether. Dry and weigh as HgCl. Factor for formic acid is 0.0977.

Notes.—The following suggestions[1] will assist in obtaining successful results with the method: The apparatus may be set up as in an ordinary steam distillation, practically the same as in Fig. 23. A liter flask, two-thirds filled with water and containing a few pieces of pumice which have been heated red hot and dropped into the water to ensure steady boiling, serves as a steam generator. The steam passes from this into a 330-cc. round-bottom flask with a short wide neck, containing the sample to be tested. The delivery tube from this flask is of rather large bore and leads to a liter pear-shaped flask with a neck not over 3 in. long. This flask contains the carbonate mix; the bottom of the entry tube leading into it should be blown into a bulb and pierced with 6 or 7 small holes (by means of the sharp red hot end of a small file) arranged in a ring a short distance from the very bottom. The entering steam is thus divided into many small bubbles and mixes thoroughly with the suspended carbonate. From this flask a delivery tube leads to a condenser, simply for convenience and to judge the volume of the distillate. Restrict the volume of filtrate and washings from the carbonate mix to about 140 cc. Evaporate, if necessary, to this volume before acidulating. In heating the reaction mixture, immerse in the bath to the level of the liquid, but not over this level, and filter soon after the heating is completed. The distillation requires about 2 hours.

[1] W. A. Bender: *Bur. of Chem., Bull.* **162,** p. 78.

In this method, the greater part of the acetic acid and all of the formic acid are retained in the carbonate flask as soluble calcium salts and formic acid is determined by its reduction of mercuric chloride to the insoluble mercurous chloride. The method recovers 90 to 95 per cent. of the formic acid, the principal error being in the distillation, since the precipitation of the calomel is quantitative.

Glycerin.—*Solutions Required.*—(1) *Strong Bichromate:* Dissolve 74.56 grams dry, recrystallized potassium bichromate in water, add 150 cc. concentrated sulphuric acid, cool and make up to 1000 cc. at 20°C.; 1 cc. of this solution equals 0.01 gram glycerin. The high coefficient of expansion of this strong solution necessitates careful volumetric measurement on account of the changes in room temperature from day to day. The solution has an apparent expansion in glass of 0.0005 (or 0.5 per cent.) for each degree centigrade. By observing this correction the solution may be measured if the room temperature varies from 20°C.[1]

(2) *Dilute Bichromate.*—Introduce 12.5 cc. of the strong bichromate into a 250-cc. glass-stoppered volumetric flask, dilute with water, and make up to the mark at room temperature; 20 cc. of this solution is equivalent to 1 cc. of the strong bichromate.

(3) *Ferrous Ammonium Sulphate.*—Dissolve 30 grams of crystallized ferrous ammonium sulphate in water, add 50 cc. of concentrated sulphuric acid, cool, and dilute to 1000 cc. at room temperature; 1 cc. of this solution is approximately equivalent to 1 cc. of the dilute bichromate. Its value changes slightly from day to day and it should be standardized against the dilute bichromate whenever used.

Extraction.—Make all evaporations on a water-bath, the temperature of which is maintained between 85° and 90°C. Evaporate 100 cc. of vinegar to about 5 cc., add 20 cc. of water and again evaporate to about 5 cc. in order to expel acetic acid. Treat the residue with about 5 grams of fine sand and with 15 cc. of milk of lime (freshly prepared and containing about 15 per cent. of calcium oxide), and evaporate almost to dryness with

[1] More accurate results may be obtained, if necessary, by taking the specific gravity of the solution and using weighed quantities from a weight burette.

frequent stirring (avoid formation of dry crust or evaporation to complete dryness). Treat the moist residue with 5 cc. of hot water, rub into a homogeneous paste, and then add 45 cc. of absolute alcohol, washing down the sides of the dish to remove adhering paste and stir thoroughly. Heat the mixture on a water-bath, with constant stirring, to incipient boiling, and decant the liquid through a 12.5-cm. fluted filter into a porcelain dish. Wash the residue twice by decantation, then repeatedly with small portions of hot 90 per cent. alcohol, transfer all of the material to the filter and continue washing until the filtrate amounts to about 150 cc., or, instead of filtering, centrifuge and wash three times. Evaporate to a sirupy consistency, add 10 cc. of absolute alcohol to dissolve the residue, and transfer to a 50-cc. glass-stoppered cylinder, using two additional portions of 5 cc. each of absolute alcohol to wash out the dish and complete the transfer. Add three portions of 10 cc. each of absolute ether, shaking thoroughly after each addition. Let stand until clear, then pour off through a filter, and wash the cylinder and filter with a mixture of one part of absolute alcohol to one and one-half parts of absolute ether, pouring the wash liquor also through the filter. If a heavy precipitate is observed in the cylinder, it is advisable to centrifuge at low speed and decant the clear liquid through a filter. Add 20 cc. of the mixture of absolute alcohol and absolute ether (1:1.5) to the precipitate in the cylinder, shake thoroughly, centrifuge and decant; repeat this process three times. Evaporate the filtrate and washings to about 5 cc. on the water-bath, add 20 cc. of water and evaporate to about 5 cc.; again add 20 cc. of water and evaporate to about 5 cc.; finally add 10 cc. of water and evaporate to 5 cc. (These evaporations are necessary to remove all the ether and alcohol and when conducted at 85° to 90°C. there is no loss of glycerin up to 50 per cent. concentration.) Transfer the residue with hot water to a 50 cc. volumetric flask, cool, add silver carbonate freshly precipitated from 0.1 gram of silver sulphate with a dilute solution of sodium carbonate, shake occasionally, and allow to stand 10 minutes; then add 0.5 cc. of lead subacetate solution, shake occasionally, and allow to stand 10 minutes; make up to the mark, shake well, filter, rejecting the first portion of filtrate, and pipette off 25 cc. of the clear filtrate into a 250-cc. glass-stoppered volumetric

flask. Add 1 cc. of concentrated sulphuric acid to precipitate the excess of lead (which otherwise would subsequently combine with part of the standard bichromate and cause an error). Then determine the glycerin by the following method:

Determination.[1]—Introduce into the 250-cc. flask, containing the 25-cc. purified glycerin solution, the strong bichromate solution, carefully add 24 cc. of concentrated sulphuric acid, rotating flask gently to mix contents and avoid violent ebullition, then place in boiling water-bath for exactly 20 minutes. Remove flask from bath, dilute at once, cool, and make up to mark at room temperature. A slightly more accurate oxidation may be obtained by adding only 15 cc. of concentrated sulphuric acid and continuing the digestion for at least 2 hours in a boiling water-bath.

Standardize the ferrous ammonium sulphate solution against the dilute bichromate solution by introducing from the respective burettes approximately 20 cc. of each of the two solutions into a beaker containing 100 cc. of distilled water. Complete the titration, using potassium ferricyanide solution as the indicator on a porcelain spot plate. From this titration, calculate the volume (F) of ferrous ammonium sulphate equivalent to 20 cc. of the dilute solution and also, therefore, to 1 cc. of the strong bichromate solution.

In place of the dilute bichromate solution now substitute a burette containing the oxidized glycerin with the excess of bichromate solution, and ascertain how many cubic centimeters of it are equivalent to (F) cubic centimeters of the ferrous ammonium sulphate solution, and also, therefore, to 1 cc. of the strong bichromate. Then 250 divided by this last equivalent equals the number of cubic centimeters excess of the strong bichromate solution present in the 250-cc. flask after oxidation of the glycerin.

The number of cubic centimeters of strong bichromate added, minus the excess found after oxidation, multiplied by 0.01 gram, equals the weight of glycerin in the 25 cc. of purified solution used in the determination; this result, multiplied by 2, gives the weight of glycerin in grams per 100 cc. of the vinegar.

[1] Hehner method, modified by Richardson and Jaffé (*J. Soc. Chem. Ind.*, **1898,** 330) and by Ross (*Bur. of Chem., Bull.* **137,** p. 61).

Lead Precipitate.—To 10 cc. of the vinegar in a test-tube, add 2 cc. of normal lead acetate (20 per cent. solution), shake and let stand for half an hour. Describe the precipitate as light, medium or heavy.

Note.—It was formerly supposed that the precipitate with lead acetate was due to malic acid, and that this being a characteristic component of cider vinegar, the lead test afforded a convenient and ready method for testing the genuineness of the article. The work of several investigators, however, has shown that the test is by no means a characteristic one. A similar precipitate is given by tartaric and phosphoric acids. Further, as both Browne[1] and Van Slyke[2] have pointed out, although malic acid is present in apple juice, the amount decreases when the juice is fermented and may even disappear altogether.

Interpretation of Results

The Federal standard for cider vinegar is as follows:[3]

"Vinegar, cider vinegar, apple vinegar, is the product made by the alcoholic and subsequent acetous fermentation of the juice of apples, is levo-rotatory, and contains not less than four (4) grams of acetic acid, not less than one and six-tenths (1.6) grams of apple solids, of which not more than fifty (50) per cent. are reducing sugars, and not less than twenty-five hundredths (0.25) gram of apple ash in one hundred (100) cubic centimeters (20°C.); and the water-soluble ash from one hundred (100) cubic centimeters (20°C.) of the vinegar contains not less than ten (10) milligrams of phosphoric acid (P_2O_5) and requires not less than thirty (30) cubic centimeters of decinormal acid to neutralize its alkalinity."

This standard is decidedly liberal and it is not especially difficult for manufacturers to prepare a spurious article which will conform to the figures given. Leach and Lythgoe,[4] from the examination of 22 samples of known purity, suggest a standard of purity which is somewhat more rigorous. They state that pure cider vinegar should contain at least 4.5 per cent. of ac-

[1] *J. Am. Chem. Soc.*, **1903,** 24.
[2] *New York Agr. Expt. Station, Bulletin* **258.**
[3] *U. S. Dept. of Agr., Office of the Secretary, Circular* **19.**
[4] *J. Am. Chem. Soc.*, **1904,** 375.

etic acid and 2 per cent. of cider vinegar solids. The ash should constitute at least 6 per cent. of the solids. The alkalinity of 1 gram of ash should be equivalent to at least 65 cc. of tenth-normal acid. The reducing sugars should not exceed 25 per cent. of the solids. The polarization, expressed in terms of 200 mm. of undiluted vinegar, should lie between − 0.1° and − 4.0° Ventzke.

Balcom[1] has compiled and calculated to a uniform basis the published results of analyses (about 100) of vinegars of known purity. These are shown in the following table:

TABLE LXVI.—TYPICAL VINEGARS

(Grams per 100 cc.)

	Total acid	Total solids	Non-sugar solids	Reducing sugars in solids, per cent.	Total ash	Alkalinity of water-soluble ash	Ash in non-sugar solids, per cent.	Phosphoric acid (Mgm. of P_2O_5)			Polarization direct, ◦V
								Soluble	Insoluble	Total	
Maximum	7.96	4.52	2.89	45.0	0.52	56.0	26.5	39.9	32.0	64.2	−3.6
Minimum	3.29	1.37	1.26*	5.6	0.20	21.5	11.2	6.7	4.3	15.1	−0.2
Average	4.94	2.54	1.90	19.6	0.367	35.7	18.8	17.3	12.0	29.3	−1.46
A	4.31	0.18	0.16	11.1	0.016	1.5	10.0	0.2	1.5	1.7	+0.6
B	4.51	1.27	0.80	37.0	0.20	17.5	25.0	5.5	7.4	12.9	−0.3
C	4.72	2.15	1.05	51.2	0.28	37.0	26.7	8.7	10.4	19.1	.....
D	4.46	2.11	0.91	56.9	0.29	33.0	31.9	11.5	10.8	22.3	−1.0
E	4.66	2.09	1.73	17.0	0.56	11.1	32.4	1.8	15.2	17.0	0.0

* Abnormally low; the next lowest values are 1.35, 1.35 and 1.39. These four are the only ones out of 63 analyses found to be below 1.40.

The maximum and minimum figures given illustrate very well the important fact that owing to the variable amounts of sugar in the original juice and the fermented product, the non-sugar solids are much more nearly constant than the total solids. For the same reason, the percentage of ash in the non-sugar solids is a much more valuable factor than the ash in the total solids in determining adulteration.

Sample A is an uncolored spirit or distilled vinegar. Vinegars of this type are frequently colored with caramel and sold as "malt vinegar" or used to adulterate cider vinegar.

B is a mixture of equal volumes of a spirit vinegar (*A*) with

[1] *Bur. of Chem., Bull.* **132,** p. 96.

cider vinegar. In comparison with the average values for cider vinegar, note the lowering in total solids as well as the reversal of the ratio between soluble and insoluble phosphoric acid. The most marked change, however, comes in the lowering of the non-sugar solids.

C is a very similar mixture of cider and spirit vinegar, except that boiled cider has been added to bring up the total solids. The adulteration is still shown, however, by the low value of the non-sugar solids, taken in connection with the abnormally high per cent. of sugar in the total solids. The relation between soluble and insoluble phosphoric acid remains the same as in sample *B*.

D is a commercial sample, the analysis of which shows it to be of the same nature as *C*. Numerous samples of this kind are on the market, which are undoubtedly mixtures of cider vinegar and dilute acetic acid, probably in the form of distilled vinegar, to which has been added some material high in sugar and ash, believed to be in most cases such substances as boiled cider, apple jelly or unfermented apple juice.

E is a sugar vinegar made from New Orleans molasses by the generator method, a type of vinegar which is being made in increasing quantities to replace the colored spirit vinegar.

The figures show how closely it resembles cider vinegar, the chief difference being in the high ash, low alkalinity of the ash and very low soluble phosphoric acid.

As a result of an extended investigation into the manufacture of cider vinegar by the generator process, in which over a thousand samples were analyzed at the factories,[1] the most important determinations for judging the purity of a vinegar were found to be: Total solids, reducing sugars after evaporation, non-sugars, ash, acidity, glycerin and formic acid. It was found that the non-sugars were fairly constant and varied between 1.5 and 2.6 grams per 100 cc.; further, that the percentage of ash in the non-sugars, which is readily calculated when both ash and non-sugars are known, was quite constant, varying between 13 and 19 per cent. The lowest amount of glycerin found in any sample was 0.24 gram per 100 cc., while the highest

[1] Tolman and Goodnow: *J. Ind. Eng. Chem.*, **1913**, 928; Bender: Recent Methods for the Detection of Adulterated Vinegar.

was 0.40 gram. The highest acetic acid found was 6.3 grams per 100 cc. Commercial acetic acid contains from 0.5 to 1.5 per cent. of formic acid; distilled vinegar contains practically none. Cider vinegar, when tested for formic acid by the method described on page 367 gives a slight precipitate corresponding to less than 7 mgm. of formic acid per 100 cc., due possibly to small amounts of volatile reducing bodies retained by the calcium carbonate. The amount found in samples adulterated with commercial acetic acid varies from 15 to 60 mgm. per 100 cc.

Table LXVII comprises some analyses made in the writer's laboratory of cider vinegar and adulterants from known sources.[1]

TABLE LXVII.—ANALYSES OF VINEGAR AND ADULTERANTS

Grams in 100 cc.

	1	2	3	4	5	6	7	8
Specific gravity	1.0520	1.0297	1.0458	1.0133	1.0244	1.0195	1.0212	.....
Total solids	12.94	6.66	5.21	2.02	2.85	2.38	2.87	54.96
Red. sugar after evap	8.57	4.42	1.53	0.25	0.57	0.62	0.73	46.94
Polarization, °V	−6.85	−8.38	−7.39	−0.20	−1.12	−1.4	−1.3	−189.6
Ash	0.35	0.19	0.23	0.28	0.33	0.35	0.37	1.38
Soluble ash	0.27	0.16	0.19	0.24	0.27	0.29	0.30	1.16
Alk. sol. ash (cc. $\frac{N}{10}$ acid)	7.83	13.8	15.3	27.5	33.9	27.2	33.3	88.0
Sol. P_2O_5 (mgm. in 100 cc.)	11.8	0.84	6.05	12.65	8.04	11.20	11.7	49.0
Insol. P_2O_5 (mgm. in 100 cc.)	13.2	11.8	8.98	9.55	13.9	15.6	24.7	36.0
Total acid	0.51	0.21	0.48	4.33	7.87	5.67	6.60	.....
Pentosans	0.23	0.36	0.36	0.14	0.19	0.40	0.36	.....
Vol. red. substances	.....	.....	.....	0.07	0.22	0.31	0.24	.....

1. Pure apple juice pressed in the laboratory from Baldwin apples.
2. Apple waste extract made by boiling the skins and cores of the apples used in No. 1.
3. Commercial apple waste extract.
4. Pure vinegar made in the laboratory from No. 1. Fermentation not carried so far as in commercial products.
5. Pure cider vinegar made by the commercial generator process from russet apples.
6. Second pressings from the stock used in No. 5.
7. Third pressings from the stock used in No. 5.
8. Boiled cider.

Crawford[2] has pointed out that the volatile copper reducing substance, referred to under the determination of reducing

[1] L. F. Hamilton: Thesis, Mass. Inst. Technology, 1914.

[2] *J. Ind. Eng. Chem.*, **1913,** 845.

sugars on page 366, is sufficiently characteristic of cider vinegar to make it a valuable constant in determining the purity of a sample. The amount of this volatile reducing substance, calculated as invert sugar, is found to be quite uniform, running from 0.111 to 0.149 gram per 100 cc., when reduced to a basis of 4 per cent. acidity. Distilled vinegar contains from a trace to 0.0026 gram per 100 cc. under similar conditions. The determination is made by diluting 50 cc. of the sample to 250 cc. and distilling 200 cc. Neutralize the distillate, make up to 250 cc., and determine the copper reducing power in 50 cc., using the Munson and Walker method (page 237) and expressing the result as invert sugar on a basis of 4 per cent. acidity.

The determination of pentosans and of alcohol precipitate is of considerable value in showing vinegar made from apple waste or second-pressings cider. In vinegar made from fresh and high-grade material, the pentosans and alcohol precipitate will rarely exceed 0.20 gram in 100 cc.

The following illustration of the method of judging adulteration by a critical consideration of the analytical results is given by Bender.[1]

	As found	Figured back to glycerin, 0.24
Acid as acetic	4.60	9.20
Solids	2.04	4.08
Reducing sugars	0.90	1.80
Ash	0.33	0.66
Ash in non-sugars, per cent	28.9	28.9
Glycerin	0.12	0.24
Soluble P_2O_5, mgm. per 100 cc.	12.0	24.0
Insoluble P_2O_5, mgm. per 100 cc.	8.0	16.0
Alkalinity of soluble ash, cc. $\frac{N}{10}$ acid	32.0	64.0
Polarization, °V.	−1.2	−2.4
Sugar in solids, per cent	44.1	44.1

According to the results as found by analysis and given in the first column, the sample would have to be considered a pure cider

[1] *Loc. cit.*

vinegar if judged by the standard on page 372. It does not, however, as can be shown, correspond to the definition incorporated in the standard that cider vinegar is the fermented product of pure apple juice.

In the second column, the results have been re-calculated from the standpoint of the glycerin, the value of which is found to be 0.12, instead of 0.24, the minimum value reported for pure cider vinegar. This reduction must have come, of course, from dilution with water or some substance containing no glycerin. The acid content of the original vinegar before dilution must, then, have been twice 4.6 or 9.2 grams in 100 cc. This is an impossible result for cider vinegar and indicates at once that the diluting substance was either distilled vinegar or dilute acetic acid. The ash also is abnormal. A reduction of one-half that of normal vinegar would give about 0.16 gram per 100 instead of the 0.33 that is shown. That the ash has been largely increased by addition is further shown by the fact that nearly 29 per cent. of the non-sugars is ash, as compared with the known maximum for normal vinegar of 0.19 per cent.

In the same way the phosphoric acid, calculated back to the original sample shows 40 mgm. per 100 cc., a result considerably in excess of the value for normal vinegar. Valuable information can also be secured from the determination of reducing sugars. The normal value is from 0.3 to 0.8 gram per 100 cc., and in the case under consideration the expected value would be one-half these or 0.15 to 0.4. It is evident, then, that from 0.5 to 0.7 gram of the sugar present has been supplied in some extraneous form, such as boiled cider. From the known composition of the latter material, this would involve adding about 0.12 gram of non-sugars. If to this 0.12 we add the 0.17 of added ash and subtract the sum from the 1.14 gram found for the non-sugars, we get the figure 0.85 which represents the true non-sugar content of the original vinegar, a value just about one-half the normal content.

From all these considerations, it can be alleged that the sample is adulterated in that distilled vinegar or dilute acetic acid together with foreign mineral matter and some substance high in reducing sugar, has been substituted in large part for cider vinegar. If the analysis had shown considerable amounts of

formic acid, as above 20 mgm. per 100 cc., the analyst would be warranted in concluding that dilute acetic acid was the adulterant rather than distilled vinegar.

In making comparisons, as above, the values taken should be such as to give the sample the benefit of every doubt. While the comparison works out very nicely from the standpoint of the glycerin content, as illustrated, the calculation can be based on any other determination, in which the result is reasonably constant for pure cider vinegar and in which none of the substance in question could be introduced by the adulteration, basing the calculation, of course, on minimum values. For example, the determination of volatile reducing substances, as suggested by Crawford, page 375, might very well be used in the same way.

As the methods for the adulteration of vinegar became more refined and exact, in that they are based on more thorough knowledge of the analytical variations of the genuine article, it is evident that the judgment of the purity of a suspected sample cannot be usually based on any one determination. A fairly complete analysis is essential.

Selected References

Bender.—Recent Methods for the Detection of Adulterated Vinegar.

Browne.—Chemical Study of the Apple and its Products. Pa. Dept. of Agr., Bull. 58.

Frear.—Vinegar. U. S. Dept. of Agr., Bur. of Chem., Bull. 65.

Tolman and Goodnow.—Study of Cider Vinegar made by Generator Process. J. Ind. Eng. Chem., 1913.

CHAPTER X

FLAVORING EXTRACTS

The flavoring extracts are, in general, solutions in alcohol of the proper strength of the odorous principles obtained from aromatic plants. They may be prepared by direct maceration in alcohol of the desired portion of the plant, as in the case of vanilla; or by simple solution in alcohol of the essential oil obtained from the plant by distillation or pressure, as in the commercial method for making lemon extract. They are of considerable interest to the food analyst because of the different analytical methods that their examination involves, and because of the ingenious substitutes and forms of adulteration that their relatively high price causes.

Three typical examples will be considered: vanilla, lemon and ginger.

Extract of Vanilla

Vanilla extract is prepared from the fruit of the *Vanilla Planifolia*, a climbing plant of the orchid family and a native of Mexico, from which country the most highly prized vanilla is still obtained. The dried and cured fruits, or so-called "vanilla beans," as they appear in commerce, are long and slender, of a lustrous brown color, and often covered with fine needle-like crystals of vanillin.

Besides the Mexican beans, other and important varieties are the Bourbon (from the Island of Reunion), Seychelles, Comores, Madagascar, and Tahiti. The last-named variety is of inferior quality and yields a low-grade extract, being used chiefly for blending and "body." The Tahiti vanilla brings much the lowest price in the market, selling for less than dollar a pound, while the best Mexican varieties will bring from five to eight.

Preparation of Vanilla Extract.—The customary method of preparing the extract is by maceration of the chopped beans in

alcohol of about 50 per cent. strength. Sugar is sometimes added both to sweeten the product and by osmosis to assist the extraction. Some manufacturers use glycerin for the same purpose.

A typical formula is that given in the United States Pharmacopœia[1] for the preparation of "Tincture of vanilla."

Vanilla, cut into small pieces and bruised, 100 grams; sugar, in coarse powder, 200 grams; and alcohol and water, each in sufficient quantity to make 1000 cc.

Mix 650 cc. of alcohol with 350 cc. of water; macerate the vanilla in 500 cc. of the mixture for 12 hours; then drain off the liquid and set it aside. Transfer the vanilla to a mortar, beat it with the sugar into a uniform powder, then pack it in a percolator and pour upon it the reserved liquid. When this has disappeared from the surface, continue the percolation by gradually pouring on sufficient menstruum to make 1000 cc. of tincture.

Many manufacturers use a more dilute alcohol than is prescribed in the method of the Pharmacopœia, although a strength less than 45 per cent. is impracticable on account of yielding a gelatinous or gummy extract. The time of extraction is usually much longer, being extended in many cases to several months, in order to exhaust the bean and especially to remove the color, which is slow of extraction. Occasionally, potassium bicarbonate may be added to assist in dissolving the fragrant resin and thus economize in alcohol.

Forms of Adulteration.—The adulterations of vanilla extract may consist in the substitution of some other natural extract which has a flavor resembling that of vanilla or in the use of an entirely factitious product so prepared as to resemble in appearance and in odor the genuine article. Typical of the first form are mixtures having as their base extract of Tonka bean, prepared from the seeds of a tropical tree, *Coumarouna odorata*. This extract resembles true vanilla in its general appearance and has a somewhat similar though coarser odor. Artificial extracts are usually made up of weak alcohol containing synthetic vanillin and coumarin, the active principles of vanilla and tonka respectively (see page 382), sometimes with an addition of prune juice or similar material to give more body to the preparation. Caramel is used for color.

Weak tinctures of true vanilla, obtained by macerating again

[1] Eighth Revision, p. 484.

the residues from the preparation of high-grade extracts, are sometimes used as a basis for adulterated extracts. These are usually reinforced or strengthened by the addition of synthetic vanillin or coumarin, or both, and colored with caramel or occasionally coal-tar colors.

Methods of Analysis

Alcohol.—Measure out 25 cc. of the sample, add 50 cc. of water, and distill 50 cc. as directed on page 416. The distillate may be tested for the presence of methyl alcohol, if desired, by the method described on page 428.

Vanillin and Coumarin.—*Gravimetric Method.*[1]—Weigh 50 grams of the extract into a 250-cc. beaker with marks showing volumes of 80 cc. and 50 cc.; dilute to 80 cc. and evaporate to 50 cc. in a water-bath kept at 70°C. Dilute again to 80 cc. and evaporate to 50 cc. Transfer to a 100-cc. flask, rinsing the beaker with hot water, add 25 cc. of standard lead acetate solution (80 grams of pure crystallized lead acetate per liter), make up to the mark with water, shake and allow to stand 18 hours at 37° to 40°C. in a bacteriological incubator, water-bath provided with a thermostat, or other suitable apparatus. Filter through a small dry filter and measure 50 cc. of the filtrate into a separatory funnel. Save the remainder of the filtrate for the determination of lead number and color ratios as described below.

To the portion in the separatory funnel add 20 cc. of ether[2] and shake. Draw off carefully the aqueous liquid together with any ether emulsion that may have formed, and transfer the clear ether solution to another separatory funnel. Shake the aqueous liquid three times more with ether in the same manner, using 15 cc. each time.

Shake the combined ether extract four or five times with 2 per cent. ammonia (one part of strong ammonia to fifteen parts of water), using 10 cc. for the first shaking and 5 cc. for each subse-

[1] Hess and Prescott: *J. Am. Chem. Soc.*, **1899,** 256; Winton and others: *J. Am. Chem. Soc.*, **1902,** 1128; **1905,** 719; *Bur. of Chem., Bull.* **132,** p. 109; *Bull.* **137,** p. 120.

[2] For all extractions of the vanillin or coumarin use ether which has been washed twice with water to remove alcohol.

quent shaking. In drawing off the ammoniacal solution, take care that none of the ether solution passes along with it. Reserve the ammoniacal solution for the determination of vanillin.

Transfer the ether solution to a weighed dish and allow the ether to evaporate at room temperature. Dry in a sulphuric acid desiccator and weigh. If the residue is coumarin it will be recognized by its characteristic odor resembling "sweet grass" and if not perfectly white, should be purified by treating with three or four portions of petroleum ether (boiling point 30° to 40°C.). Stir with each portion for 15 minutes, decant carefully finally dry and re-weigh the dish, taking the coumarin as the loss in weight. Evaporate the petroleum ether in a porcelain dish and confirm the presence of coumarin by Leach's test (see below).

Add to the ammoniacal solution 10 per cent. hydrochloric acid to slightly acid reaction. This should be done without delay, as the ammoniacal solution on standing grows darker slowly with a loss of vanillin. Cool and shake out in a separatory funnel with four portions of ether, as described for the first ether extraction. Evaporate the ether solution at room temperature in a weighed dish, dry over sulphuric acid and weigh. The residue should be pure vanillin, free from any appreciable amount of color and with a melting point of 80°C.

Notes.—The method given for the separation of vanillin and coumarin is based on the difference in their chemical constitution. Vanillin, *m*-methoxy-*p*-oxybenzaldehyde, is found in the vanilla bean up to 3 per cent., but is now made artificially on a considerable scale by the oxidation of the eugenol of oil of cloves with alkaline potassium permanganate. Coumarin is the anhydride of coumaric acid, and may also be prepared synthetically. On account of the aldehydic nature of the vanillin compound, the separation by dilute ammonia is possible, the aldehyde-ammonia compound of vanillin being readily soluble in water while the coumarin remains wholly in the ether.

Although the method as outlined should theoretically give pure residues of vanillin and coumarin, this is seldom the case, the residues, except with entirely artificial extracts, being contaminated with gummy or resinous matter. The results obtained by weighing the residues directly, without purification

are consequently usually too high, the error sometimes amounting to 0.04 per cent. If the weighed residues of vanillin and coumarin are discolored, indicating impurities, they should be purified as described above under the determination of coumarin, except that in the case of vanillin the treatment with low boiling petroleum ether should be more prolonged, not less than fifteen extractions being made with the boiling solvent. This repeated treatment is necessary on account of the gummy character of the impurities, which prevents the ready solution of vanillin or coumarin with which they are intimately mixed. The proximity of a flame should, of course, be carefully avoided during this operation.

A simpler and less tedious method of purifying the residues is by sublimation, as suggested by Hiltner.[1] To do this, heat the residue, which has been dried over sulphuric acid and weighed, in an oven at 105°C. for 1 or 2 hours, or until its weight is constant. The loss in weight is vanillin or coumarin as the case may be. If it be desired to test the purity of the volatile matter, the dish may be heated cautiously at first on a hot plate, the sublimate condensed on a cool watch-glass and examined by appropriate tests. The dish and residue should then be placed in the oven as above in order to complete the volatilization. Vanillin and coumarin volatilize completely at 105°, leaving the gummy matter unchanged. The purity of the vanillin may also be determined by the colorimetric method (page 384).

The residue, or the sublimate obtained in the volatilization method, should be subjected to qualitative tests in order to show that it is vanillin or coumarin. In the case of the vanillin, a small amount of the residue dissolved in 2 drops of concentrated hydrochloric acid, should give a pink color upon the addition of a crystal of resorcin.

Also a portion of the vanillin dissolved in 2 or 3 drops of ether and allowed to evaporate spontaneously on a microscope slide should show a characteristic appearance with polarized light. The vanillin crystallizes in slender needles, forming star-shaped clusters. These give a brilliant play of colors with crossed Nicols, even without the selenite plate.

The coumarin residue should have the characteristic "sweet

[1] *Bur. of Chem., Bull.* **152,** p. 135.

grass" odor of coumarin, and give a positive reaction with iodine.[1] To make the iodine test, add a few drops of water to the residue, warm gently and transfer to a white porcelain dish. Add a few drops of a solution of iodine in potassium iodide, and stir with a glass rod. If coumarin is present, a brown precipitate will form and gradually collect in dark green clots. Care should be taken that the solutions are not too dilute, since the test is not one of extreme delicacy.

If vanillin only is to be determined it will probably be found a saving of time to employ the colorimetric method given below or the original extract may be tested for coumarin by Wichmann's method (page 386), and if this is found to be absent, the determination of vanillin may be simplified by evaporating and weighing the ether extract without treating it with ammonia and hydrochloric acid.

According to Leach,[2] if coumarin only is to be determined good results may be obtained by making the dealcoholized original sample slightly alkaline with ammonia, extracting it with three or four portions of chloroform in a separatory funnel, and evaporating the combined chloroform extract in a tared dish at a temperature not over 60°C.

If this procedure is carried out, however, it will be found necessary to exercise great care to prevent the formation of an obstinate emulsion with the chloroform. If such an emulsion should form, it can be broken by a suitable centrifuge or helped by stirring the chloroform layer with a loop of stout wire. With any except entirely artificial extracts, it will be necessary to purify the coumarin as directed above.

Colorimetric Determination of Vanillin.[3]—*Reagent.*—To 10 grams of pure sodium tungstate and 20 grams of phosphomolybdic acid (free from nitrates and ammonium salts) add 100 grams of sirupy phosphoric acid (containing 85 per cent H_3PO_4) and 700 cc. of water; boil over a free flame for 1½ to 3 hours; then cool, filter if necessary, and make up with water to 1 liter. An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

[1] Leach: Food Analysis, Third Ed., p. 867.
[2] *Loc. cit.* p. 866.
[3] Folin and Denis: *J. Ind. Eng. Chem.*, **1912**, 670.

Process.—Measure 5 cc. of the vanilla extract with a pipette into a 100-cc. flask, add 75 cc. of water, 4 cc. of lead acetate solution (containing 5 per cent. basic and 5 per cent. neutral lead acetate) and make up to 100 cc.

Filter rapidly through a fluted filter paper and transfer 5 cc. of the filtrate with a pipette to a 50-cc. flask. In another 50-cc. flask place 5 cc. of the standard vanillin solution (0.1 gram per liter); then add 5 cc. of the phosphotungstic-phosphomolybdic reagent to each flask, allowing it to run down the neck of the flask in order that any adhering vanillin may be washed down. After shaking, allow the flask to stand for 5 minutes and then fill to the mark with saturated sodium carbonate solution. Invert the flask several times to mix the contents, and allow to stand for 10 minutes for complete precipitation of the sodium phosphate. Filter rapidly through fluted filters and compare the color of the resultant deep blue solutions with a Duboscq or other colorimeter. The standard solution is best placed at 20 mm., if using the Duboscq instrument, as experience has shown that the best reading is obtained at this point. Do not read the solutions unless they are absolutely clear. The readings should be made without any great delay, since the color darkens slowly for about an hour after adding the alkali.

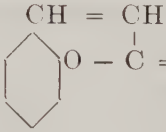
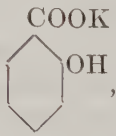
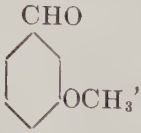
Notes.—The exact reaction which occurs is not definitely known but is supposed to be the reduction of the highly complex reagent by the vanillin (also by other phenolic compounds) in acid solution, and the production of blue salts by the addition of a weak alkali to the reduced substance.

Before taking readings, the correctness of the colorimeter should be tested by placing the standard solution in both tubes and noting the readings, which should agree within 1 per cent. A slight disagreement may sometimes be corrected by varying somewhat the relative position of the instrument and the source of light. Readings should not be taken as final if the variation between the standard and the unknown is greater than 20 per cent., but in such a case the determination should be repeated, using a fresh standard and a proportionately greater or less amount of the filtrate from the lead precipitate.

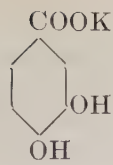
Apart from serving as a direct determination of the vanillin, the colorimetric method may be used as a check on the gravi-

metric method or to replace the tedious process of purification. To do this, dissolve the impure vanillin residue (page 383) after drying and weighing, in the smallest possible quantity of 25 per cent. alcohol, transfer to a graduated flask and make up with water to such a concentration that 10 cc. of the solution shall contain approximately 1 mgm. of the residue. To 5 cc. of this solution, add 5 cc. of the phosphotungstic-phosphomolybdic reagent, as described above, and compare with a standard prepared in the same way.

Wichmann Test for Coumarin.[1]—Dilute 25 cc. of the extract with an equal volume of water, slightly acidify, if alkaline, with sulphuric acid and distil to dryness. To the distillate, which will contain the vanillin and coumarin, add 15 to 20 drops of potassium hydroxide solution (1 : 1), evaporate over a flame to 5 cc., transfer to a test-tube and heat over a small flame until the water is expelled and the residue just fuses to a nearly colorless mass. Cool the melt and dissolve it in a few cubic centimeters of water, transfer to a 50-cc. distilling flask and acidify slightly with 25 per cent. sulphuric acid. Finally distil the solution (which should not exceed 10 cc.) into a test-tube containing 4 or 5 drops of neutral 0.5 per cent. ferric chloride. If coumarin was present in the original extract, a purple color will develop, the intensity being proportional to the amount of coumarin.

Notes.—When coumarin,  , is fused with potassium hydroxide, potassium salicylate,  , is formed. Vanillin,  , under the same conditions forms potassium

[1] *Bur. of Chem., Circular* **95.**

protocatechuate,  . The usual test for salicylic acid with ferric salts cannot be applied directly to an aqueous solution of the fusion on account of the somewhat similar reaction given by protocatechuic acid, hence the separation of the volatile salicylic acid by distillation. Some other substances, saccharin, for instance, would be converted into salicylic acid under similar conditions, but are not likely to be present.

Care should be taken to control the temperature rather closely in fusing the residue with potassium hydroxide. After evaporating to dryness, the residue should be heated cautiously only to the point of incipient fusion.

Normal Lead Number.[1]—Mix 10 cc. of the filtrate from the lead acetate precipitate obtained in the determination of vanillin and coumarin (page 381) with 25 cc. of recently boiled distilled water and a moderate excess of sulphuric acid. Add 100 cc. of 95 per cent. alcohol and mix again. Let stand over night, filter on a Gooch crucible, wash with 95 per cent. alcohol, dry in the water-oven, ignite at low redness for 3 minutes, taking care to avoid the reducing flame, and weigh. Run a blank under exactly the same conditions in order to determine the value of the standard lead acetate and calculate the result by the following formula:

$$P = \frac{100 \times 0.6831\ (S - W)}{5}$$

where P equals normal lead number, S equals grams of lead sulphate corresponding to 2.5 cc. of the standard lead acetate as determined in the blank analysis and W equals grams of lead sulphate obtained in 10 cc. of the filtrate from the lead acetate precipitate as described.

Notes.—If no suitable apparatus is available for keeping the solutions at 37° to 40°C., they may be allowed to stand over night at room temperature. Winton and Berry found, however, that the amount of lead precipitated is materially affected by the time

[1] Winton and Lott: *Bur. of Chem., Bull.* **132,** p. 110; Winton and Berry: *Bur. of Chem., Bull.* **152,** p. 148.

of standing after adding the standard lead acetate solution, and by the temperature of the solution while standing. The temperature of 37°C. was chosen because bacteriological incubators, kept at that temperature, are available in many laboratories.

The method is an adaptation of the one devised by Winton for determining the purity of maple products. (See page 281.) The use of basic lead acetate, however, is objectionable in the case of vanilla extract, since it carries down vanillin, thus precluding the possibility of determining this in the same sample. Neutral lead acetate is free from this objection.

Detection of Caramel.—The test for caramel depending upon its insolubility in paraldehyde, as described on page 56, may be used to show its presence in vanilla extracts if preceded by the treatment with zinc hydroxide. Positive results should, however, be interpreted with caution as indicating the presence of caramel, because with some low-grade natural extracts, especially those made from Tahiti beans, some resinous extractive matter is thrown down by paraldehyde, giving a flocculent precipitate. A positive result should not be declared unless a dark brown precipitate, adherent to the walls of the tube, is obtained. Unless considerable experience has been had with the paraldehyde test better results will probably be obtained with the methods described below, especially the first one.

(*a*) **Marsh Test.**[1]—Evaporate 25 cc. of the extract until the greater part of the alcohol has been removed. Dilute the remainder with water and alcohol, using 26.3 cc. of 95 per cent. alcohol (equivalent to 25 cc. of absolute alcohol) and making up to the mark in a 50-cc. flask with water. Transfer 25 cc. of this solution to a separatory funnel; add 25 cc. of the Marsh reagent and shake, not too vigorously to avoid emulsification. Allow the layers to separate but still to remain in the funnel, and repeat the shaking twice more. After the layers have separated clearly, draw off the lower layer into a 25-cc. cylinder, and make up to volume with 50 per cent. (by volume) alcohol. Compare in a colorimeter with the remaining 25-cc. portion (which has not been extracted with the reagent), filtering both solutions before placing in the colorimeter. Express the result as *per cent. of color insoluble in amyl alcohol.*

[1] *Bur. of Chem., Bull.* **152,** p. 149.

The Marsh reagent is prepared as follows:

Mix 100 cc. of amyl alcohol, 3 cc. of sirupy phosphoric acid, and 3 cc. of water; shake before using. If the reagent becomes colored on standing, the amyl alcohol should be re-distilled over 5 per cent. phosphoric acid.

Note.—The method is based on the fact that the natural coloring matter of the vanilla bean is largely soluble in acid amyl alcohol, while caramel is almost completely insoluble. Hence, in caramel-colored extracts there will generally be a preponderance of color insoluble in amyl alcohol, while in genuine vanilla the proportion of insoluble color rarely exceeds 35 per cent. and is usually below 25 per cent. See page 486 for the application of the method to determine the color of whiskey.

(*b*) **Lead Acetate Test.**—Evaporate a portion of the sample to about a third of its volume to remove alcohol, dilute to the original volume with water, add an excess of neutral lead acetate and filter. If caramel is present the filtrate will be deep yellow brown to brown; if the extract is pure the filtrate will be colorless to pale yellow.

If the vanillin and coumarin have been determined by the gravimetric method described on page 381, the presence of caramel may be inferred even better from the color of the lead acetate filtrate obtained in that method. In natural products, irrespective of the nature of the solvent, the filtrate will be of a light straw color seldom exceeding a reading on the Lovibond tintometer of 1.5 red and 6.0 yellow, in a 1-in. cell.

To those experienced in the examination of vanilla extracts, the general appearance of the lead filtrate will serve to distinguish pure extracts from those colored with caramel.

(*c*) **Color Values of Extract and Lead Filtrate.**—As a logical sequence to the simple test with lead acetate described above, Winton and Berry[1] have suggested the exact determination by a tintometer of the relative proportion of red and yellow in the filtrate from the lead acetate, in order to show the presence of caramel.

1. *Color Value of the Extract.*—Pipette 2 cc. of the extract into a 50-cc. graduated flask and make up to the mark with a mixture of equal parts of 95 per cent. alcohol and water. Deter-

[1] *Loc. cit.*

mine the color value of this diluted extract in terms of red and yellow by means of a Lovibond tintometer, using the 1-in. cell. To obtain the color value of the original extract, multiply the figure for each color by 25.

For example, a reading of 0.6 red and 2.1 yellow on the diluted extract corresponds to a color value of 15.0 red and 52.0 yellow, calculated to the original extract.

2. *Residual Color after Precipitation with Lead Acetate.*—Determine the color value, in terms of red and yellow, of the filtrate from the lead acetate precipitate, obtained as on page 381, using the 1-in. Lovibond cell. Multiply the reading by 2, thus reducing the results to the basis of the original extract.

In case the actual reading of the solution is greater than 5.0 red and 15.0 yellow, as may happen if the extract is highly colored with caramel, the ½- or ¼-in. cell should be used, the readings being multiplied by 4 or 8, respectively.

Divide the figures for red and yellow respectively by the corresponding figures for the original extract and calculate the percentages of the two colors remaining in the lead acetate filtrate.

Example.—The color value of the original extract is 15.0 red and 52.0 yellow and the color value of the lead acetate filtrate, in the 1-in. cell, is 0.6 red and 2.4 yellow. The latter values, calculated to the original extract, would be 1.2 red and 4.8 yellow. Hence, $\frac{1.2}{15.0} = 8$ per cent. of the red, and $\frac{4.8}{52.0} = 9.2$ per cent. of the yellow as the percentage of color remaining in the lead filtrate.

Calculate also the ratio of red to yellow $\left(\frac{\text{yellow}}{\text{red}}\right)$ in both extract and lead filtrate.

Notes.—The method is based upon two differences between the color of caramel and the color of genuine vanilla; the former contains a much greater proportion of red to yellow than the latter, and is not precipitated so readily by lead acetate. Thus in the case of a commercial extract containing a certain amount of genuine vanilla but with the color re-inforced by caramel, the components of the color would show a higher ratio of red to yellow than would correspond to a pure extract. Further,

precipitation by lead acetate would leave in the filtrate a greater percentage of the total color, and a still greater proportion of red, because the natural vanilla color would be removed by the lead to a greater extent than the caramel. In a genuine extract there will be at least 2.2 parts of yellow to one part of red and at least 90 per cent. of the color should be removed by lead acetate.

The chief disadvantage of the method lies in the fact that it requires a special apparatus not possessed by all laboratories.

Coal-tar Colors.—These, if present, can be detected by dyeing on wool and identified by the tests described in the chapter on Colors, pages 58 to 87.

Detection of Prune Juice.[1]—To 25 cc. of the extract add 50 cc. of water and evaporate on a steam-bath to 20 cc. Filter off the precipitated resins and wash the filter with about 5 cc. of water, the washings being allowed to mix with the filtrate. When cold, transfer to a separatory funnel and extract with two 15 cc. portions of ether. Note the color of the first ether extract, the coloring matter of vanilla being quite soluble in ether, caramel and prune juice insoluble.

Draw off the aqueous liquid into a beaker and heat for a few minutes on the steam-bath to expel the last traces of ether. Transfer to a stoppered cylinder, add 1 cc. of basic lead acetate (sp. gr. 1.25), mix by inverting the cylinder two or three times and let stand until the precipitate settles.

If caramel be present the precipitate will be dark brown in color, while the supernatant liquid will be dark colored also. After the color of the precipitate and liquid have been noted, add 5 cc. of glacial acetic acid and shake; with a pure extract or one colored with caramel, the precipitate will immediately dissolve. In the presence of prune juice there will remain a reddish brown gelatinous residue much resembling the ordinary precipitate of ferric hydroxide.

Notes.—The observation as to whether or not acetic acid has dissolved all of the precipitate should be made immediately after adding the acid, as on standing for several hours or over night, traces of such precipitate begin to appear when extracts of known purity are examined.

[1] Denis: *J. Ind. Eng. Chem.*, **1911**, 254.

The results given by an unknown extract should be interpreted with caution and best after the student has gained some experience with the method on genuine samples and known mixtures. Extracts made from Tahiti beans, especially, are likely to give abnormal results in the test. Positive results should be reported only when the presence of prune juice is borne out by the results of the other determinations.

Simple Tests to Distinguish Artificial Extracts.—To distinguish between genuine extracts and artificial imitations, of which there are many on the market, is comparatively easy and several simple qualitative tests may be sufficient to identify a sample.

To one at all experienced in the examination of vanilla extract, the odor of coumarin is easily apparent in a sample containing it, even without extraction, and needs no further evidence to show its presence.

The behavior on evaporation is of distinct value. If a portion of the sample be evaporated to half its volume on the water-bath, and on dilution to the original volume remains as clear as it was at first, it can contain very little true vanilla extract, but is probably entirely artificial. Unless the resins of true vanilla are held in solution by potassium bicarbonate or ammonia, as is sometimes the case, they will precipitate when the alcohol is evaporated, and give a turbid solution. Artificial vanilla, on the other hand, having no resin to precipitate, remains clear.

Extracts which are entirely artificial may also be shown by the following simple test. Shake 5 cc. of the extract in a test-tube with an equal quantity of ether and allow the layers to separate. On another portion make a similar test, using amyl alcohol instead of ether. With genuine extracts the ether and amyl alcohol respectively will be colored from various shades of yellow to brown, due to the natural color and resin; with artificial extracts, usually colored with caramel, the ether and amyl alcohol will be colorless. It should be stated that the presence of color in the amyl alcohol or ether does not necessarily mean that the extract is pure, since it may be due to coal-tar colors, or to extractive matter other than vanilla resin, but on the other hand the absence of color indicates the absence of vanilla. With such samples the use of the word "vanilla" on the label without any qualifying term

to show that it is an imitation, is illegal, since no appreciable quantity of true vanilla can be present.

Interpretation of Results

It would naturally be expected that vanilla extract would be of varying composition because of differences in the grades and varieties of vanilla bean from which it is made, as well as variations in the solvent employed and the length of time during which maceration in the solvent is allowed to continue. With all these variations, however, the extract of true vanilla has certain pronounced characteristics which serve to distinguish it easily from artificial imitations and fairly readily from extracts made from somewhat similar natural products.

Because, presumably, of these considerable variations in raw materials and manufacturing processes, the Federal standard[1] is left exceedingly vague and indefinite, merely requiring that vanilla extract shall be prepared from the dried, cured fruit of *Vanilla planifolia*, with or without sugar and shall contain in 100 cc. the soluble matters from not less than 10 grams of the vanilla bean.

A better basis for detecting adulterations will be obtained by comparing directly analyses of genuine and adulterated extracts.

Composition of Genuine Vanilla Extract.—The most enlightening series of analyses of genuine vanilla extract is that made by Winton and Berry[2] of 75 samples prepared in the laboratory from all the standard varieties of the vanilla bean. The extracts were made by the process of the United States Pharmacopœia (see page 380) which is probably less efficient than some of the commercial methods. This, however, is by no means an injustice to the manufacturer, since any standard based on analyses of U. S. P. extracts would be rather favorable toward a commercial extract prepared by a more thorough method of extraction. A summary of these analyses is given in the following Table LXVIII.

The results obtained by the same methods on pure commercial extracts, made from various blends of vanilla beans by commercial methods rather than the U. S. P. process, are summarized in Table LXIX, page 395.

[1] *U. S. Dept. of Agr., Office of the Secretary, Circular* **19.**

[2] *Bur. of Chem., Bull.* **152,** p. 146.

TABLE LXVIII.—COMPOSITION OF PURE VANILLA EXTRACTS

Kind of bean	Vanillin gms. in 100 cc.	Normal lead no.	Per cent. of total color in lead filtrate		Ratio of red to yellow		Per cent. of color insoluble in amyl alcohol
			Red	Yellow	Extract	Filtrate	
Mexican:							
Maximum	0.20	0.68	6	9	1:3.8	1:5.6	24.4
Minimum	0.15	0.47	4	5	2.6	4.0	19.0
Average	**0.17**	**0.58**	**5**	**7**	**3.1**	**4.5**	**21.2**
Bourbon:							
Maximum	0.22	0.63	8	10	3.9	5.0	30.3
Minimum	0.13	0.44	4	5	2.3	2.8	21.3
Average	**0.18**	**0.52**	**6**	**8**	**3.2**	**3.8**	**26.6**
Seychelles:							
Maximum	0.21	0.60	7	9	3.6	5.0	29.4
Minimum	0.16	0.45	4	6	2.5	4.0	22.7
Average	**0.19**	**0.51**	**5**	**8**	**3.2**	**4.5**	**25.6**
Madagascar:							
Maximum	0.30	0.63	7	9	3.5	5.1	30.3
Minimum	0.16	0.40	4	6	2.7	3.5	23.2
Average	**0.22**	**0.50**	**6**	**8**	**3.2**	**4.5**	**26.8**
Comores:							
Maximum	0.31	0.74	8	9	3.8	5.3	30.3
Minimum	0.12	0.40	5	6	2.8	3.4	20.4
Average	**0.18**	**0.59**	**6**	**8**	**3.2**	**4.1**	**26.7**
South American:							
Maximum	0.23	0.58	6	6	3.1	4.0	29.4
Minimum	0.19	0.49	4	6	2.5	3.5	20.0
Average	**0.21**	**0.52**	**5**	**6**	**2.9**	**3.8**	**23.3**
Java:							
Maximum	0.24	0.61	7	10	3.9	4.3	35.7
Minimum	0.22	0.44	5	6	3.0	4.1	32.2
Average	**0.23**	**0.50**	**6**	**8**	**3.4**	**4.2**	**34.5**
Tahiti:							
Maximum	0.17	0.56	6	9	3.0	5.8	22.0
Minimum	0.11	0.44	4	7	2.7	3.7	16.0
Average	**0.13**	**0.50**	**5**	**8**	**2.9**	**4.8**	**18.0**
All analyses:							
Maximum	0.31	0.74	8	10	3.9	5.8	35.7
Minimum	0.11	0.40	4	5	2.3	2.8	16.0
General Average	**0.19**	**0.54**	**6**	**8**	**3.2**	**4.2**	**25.5**

TABLE LXIX.—COMPOSITION OF PURE COMMERCIAL VANILLA EXTRACTS

Sample	Vanillin, per cent.	Normal lead number	Per cent. of total color in lead filtrate		Ratio of red to yellow		Per cent. of color insoluble in amyl alcohol
			Red	Yellow	Extract	Filtrate	
1	0.20	0.50	12.0	14.0	1:2.8	1:3.0	25.6
2	0.34	0.55	7.2	8.0	3.0	3.2	22.8
3	0.19	0.50	6.3	6.7	3.0	3.0	13.3
4	0.18	0.45	5.2	5.9	3.0	3.4	17.4

These extracts were obtained[1] from reputable manufacturers, who guaranteed their purity and furnished an outline of the formulas by which they were made, as stated below:

No. 1. Extract made from the best grade of Mexican beans, with a menstruum of sugar sirup, 40 per cent. "cologne spirits" and 1 per cent. glycerin; macerated for 2 months and then percolated. The finished product was adjusted to accord with the United States standard. (See page 393.)

No. 2. Extract made from average quality Bourbon beans with sugar and 50 per cent. alcohol; macerated for 16 months and then percolated. The finished product was diluted so as to contain the extracted matter of 1 pound of beans in a gallon of the extract. (In terms of the U. S. standard this is equivalent to about 12 grams per 100 cc.)

No. 3. Extract made from Tahiti beans of average quality by the so-called "machine process" at a temperature of 100°F., requiring only about 36 hours to complete the manufacture. The menstruum was similar to No. 2. The product was diluted to about U. S. standard strength.

No. 4. Extract made according to the following formula: 29 pounds of Tahiti beans, 12 pounds prime Bourbon beans, menstruum 40 gallons of sugar and 60 per cent. alcohol; macerated for about one month, percolated, and finally diluted with water to 50 gallons, equivalent approximately to the U. S. standard.

Standards of Purity.—From the results obtained in the studies of pure vanilla extracts referred to above, the follow-

[1] Hiltner: *Bur. of Chem., Bull.* **162,** p. 82.

ing limiting values have been suggested for genuine vanilla extract:

Vanillin, not less than 0.10 nor more than 0.35 per cent.

Neutral lead number, not less than 0.40 nor more than 0.80.

Per cent. of color in lead filtrate, not more than 15 for either red or yellow.

Color insoluble in amyl alcohol, not more than 35 per cent. (25 per cent. is rarely exceeded).

Detection of Adulteration.—True extract of vanilla is characterized by its content of vanillin, varying within the limits assigned above; the presence of resins, the formation with neutral lead acetate of a flocculent gray brown precipitate, from which may be separated a yellow filtrate; and by the small proportion of color insoluble in amyl alcohol. Imitation vanilla extract, prepared ordinarily from artificial vanillin or coumarin, may contain exactly the quantity of vanillin found in genuine extracts, but is readily distinguished by the absence of resins, the scanty dark brown precipitate and brownish filtrate with lead acetate, and the high percentage of color insoluble in amyl alcohol. The presence of coumarin and vanillin over 0.35 per cent. would also be suspicious.

The detection of more skilfully prepared extracts, consisting oftentimes of low-grade Tahiti vanilla beans, re-inforced by extract of Tonka beans or prune juice, together with the required amount of vanillin to simulate a natural extract, is more difficult.

Probably the best indication of adulteration will be shown by the normal lead number since, this varies less in genuine vanilla than some of the other constants. Since the normal lead number of Tonka extract is so low (see Table LXX, page 397), this, taken in the connection with the amount of coumarin present, is of value in showing the presence of this adulterant.

The color of true vanilla seems to be the most variable constituent and the most difficult of extraction. At the same time it is possibly the constituent most difficult to imitate, especially in its relations to the other constituents. For detecting caramel, the color most commonly added, the determination of the amount of color left in the lead filtrate is of value, especially when considered in connection with the ratio of red to yellow in the ex-

tract and the lead filtrate. Caramel solution shows a lower ratio, that is, it is redder than the color of true vanilla. Even more definite is the percentage of color insoluble in amyl alcohol, which possesses the additional advantage that a tintometer is not required for the determination.

The presence of extract of prunes may be shown by the test described on page 391. As stated there, however, definite conclusions should not be drawn from the test unless some little experience has been had with the results to be obtained in pure vanilla and on prune extract.

Much help should be gained from the figures given in the following table which gives the results obtained by collaborators of the Association of Official Agricultural Chemists on various adulterated extracts.[1]

TABLE LXX.—ANALYSES OF ADULTERATED VANILLA EXTRACTS*

Sample	Vanillin, per cent.	Coumarin, per cent.	Normal lead number	Per cent. of color in lead filtrate		Ratio of red to yellow		Per cent. of color insoluble in amyl alcohol
				Red	Yellow	Extract	Filtrate	
A	None	0.25	0.11	10.0	13.0	1:3.7	1:4.8	30.8
B	0.16	0.016	0.50	7.8	11.9	3.2	3.8	47.0
C	0.17	None	0.42	9.6	14.0	2.5	3.7	52.6
D	0.14	0.04	0.19	19.5	35.5	2.0	3.6	90.9
E	0.16	None	0.39	15.1	20.2	2.6	3.4	76.9

* The samples referred to in the above table were prepared as follows:

A. Extract by U. S. P. method of Angostura Tonka beans.

B. Extract adulterated with tonka bean extract and caramel.

C. Vanilla extract (Mexican and Tahiti beans) adulterated with extract of dried prunes and synthetic vanillin, and colored with caramel.

D. A wholly factitious product, containing 25 per cent. tonka-bean extract (10 per cent. tonka beans, 20 per cent. sugar, 70 per cent. alcohol) and 75 per cent. of extract of dried prunes, to which was added synthetic vanillin and caramel.

E. Contained about 40 per cent. Tahiti-bean extract (of rather inferior grade) adulterated with synthetic vanillin, and colored with caramel.

[1] *Bur. of Chem., Bull.* **152,** p. 129.

Lemon Extract

Next in importance to vanilla as regards the extent to which it is used, is extract of lemon. This is defined in the Federal standards[1] as "the flavoring extract prepared from oil of lemon, or from lemon peel, or both, and contains not less than 5 per cent. by volume of oil of lemon." The extract was probably prepared originally by macerating lemon peel in alcohol, and a similar preparation is still listed in the U. S. Pharmacopœia under the name of "Tincture of lemon peel." The best quality of extracts on the market now, however, are practically all made by dissolving the requisite quantity of lemon oil in strong "deodorized" alcohol, the resulting product being usually, though not always, colored yellow by the addition of some coloring material. The actual color of the lemon peel is seldom employed, being fugitive to the light.

Lemon oil, which is the essential ingredient of the extract, is produced by moderate pressure from the peel or rind of the lemon, the largest quantity coming from Sicily.[2] It is a light yellow liquid, having the characteristic odor of lemons, and is composed chiefly of a terpene, *d-limonene*, and the aldehyde, *citral*. The former occurs in greatest amount, comprising approximately 90 per cent. of the oil, but the odor and value of the oil is due mainly to its citral content. Other substances present in small amounts, mainly esters and traces of other aldehydes, modify the flavor, however, so that even pure citral is by no means a satisfactory substitute for the genuine oil.

Oil of lemon is defined in the Federal standards[3] as having an optical rotation at 25°C. of not less than + 60° in a 100-mm. tube[4] and containing not less than 4 per cent. by weight of citral.

Forms of Adulteration.—In the manufacture of a genuine extract of lemon, the alcohol is the expensive part of the prepara-

[1] *U. S. Dept. of Agr., Office of the Secretary, Circular* **19.**

[2] See Chace: *J. Ind. Eng. Chem.*, **1909,** 18.

[3] *Loc. cit.*

[4] This refers to circular degrees, not degrees on the Ventzke scale.

tion, costing approximately four times as much as the oil. The producer of a cheap extract naturally seeks to reduce the cost by decreasing the proportion of alcohol. Since, however, lemon oil is nearly insoluble in dilute alcohol, such extracts must contain only very little oil, or else be made from "terpeneless" oils, as explained below. Many of the brands on the market contain so little lemon oil that it can hardly be detected by chemical tests, being often made by shaking lemon oil with weak alcohol and filtering through magnesia to remove the excess of oil, the result being nothing more than an odor of lemon. A ten-cent bottle of such extract, although colored a beautiful lemon yellow, contains materials costing but a fraction of a cent and nearly worthless as a flavor.

Since the limonene is the least valuable portion of the oil from the standpoint of flavor, and moreover, is the part of the oil which requires strong alcohol for solution, lemon oils are frequently treated so as to remove the greater part or nearly all of the limonene. This may be done by fractional distillation, or by washing the oil with dilute alcohol, by which means the citral is gradually washed out. Such oils are sold as "terpeneless," "soluble," "washed," or "concentrated" oils, and their great advantage is that they can be dissolved in weak alcohol. As intimated above, these oils are inferior in quality as compared with pure oil of lemon, and their use constitutes an adulteration unless the extract is sold under some distinctive name, as "Terpeneless Extract of Lemon" to denote that it is not a genuine extract. It is required in the Federal standards that these terpeneless extracts should contain at least 0.2 per cent. of citral, this being the amount equivalent to the presence of 5 per cent. of standard lemon oil, containing 4 per cent. of citral.

Minute quantities of highly odorous oils, as oil of lemon grass or oil of citronella, are sometimes used in cheap extracts, but these are readily distinguished by their harsh, verbena-like odor as compared with the pleasant fragrance of the genuine lemon.

Since lemons have naturally a yellow color, the popular conception of lemon extract is that it should have a similar color, so that practically all the brands on the market are colored varying shades of yellow with such colors as turmeric or coal-tar dyes. Some of the extracts containing practically no oil are

the most highly colored. Since this is an imitation of a product having a natural color of its own, the use of any color except the natural color of the lemon peel, unless its presence is disclosed on the label, constitutes an adulteration or misbranding.

Analytical Methods

Preliminary Test.—Place a small quantity of the extract in a test-tube and add eight to ten times its volume of water. If an appreciable amount of lemon oil is present a distinct cloud or turbidity will be produced, on account of the insolubility of the oil in water.

Note.—This simple test, which can be readily applied in the household, is in many cases all that is required to show the character of an extract. When no precipitate at all, or at most only a faint cloud, is produced, there can be only a trace of lemon oil present. The formation of a cloud is of course not conclusive evidence that the sample contains genuine lemon oil, but in the absence of one, lemon oil is certainly not present.

Determination of Lemon Oil.[1]—(*a*) *By Polarization.*—Polarize the undiluted extract in a 200-mm. tube at 20°C. If the instrument is graduated for the Ventzke sugar scale (see page 253), divide the reading by 3.2; if the angular degree scale is used, divide the reading, expressed in minutes, by 68. In either case, if no other optically active substance is present, the quotient is the per cent. of lemon oil by volume.

Note.—Lemon extracts occasionally contain a small amount of cane sugar, it being used to facilitate solution of the oil. In case cane sugar is present, as may readily be found by evaporating a portion of the extract, weigh out 10 grams of the sample, evaporate to dryness on the water-bath, wash by decantation with three 5-cc. portions of ether, dry and weigh. Deduct from the polarization 0.38° for each 0.1 per cent. of cane sugar found ($0.1 \times \frac{100}{26.048} = 0.38$).

(*b*) *By Precipitation.*—Measure with a pipette 20 cc. of the extract into a Babcock milk bottle (page 113), add 1 cc. of

[1] Mitchell: *J. Am. Chem. Soc.*, **1899**, 1132; *Bur. of Chem., Bull.* **107** (Rev.), p. 160.

dilute (1–1) hydrochloric acid and 25 cc. of water previously warmed to 60°C. Mix the contents of the bottle and stand it in warm water for 5 minutes. Then centrifuge for 5 minutes. Add enough warm water to bring the oil into the graduated neck of the bottle and centrifuge again for 2 minutes. Stand the bottle in water at 60° for 5 minutes and read off the per cent. of oil by volume. If the oil found is more than 2 per cent., add to the amount 0.4 per cent. to correct for the oil that does not separate. If the amount is between 1 per cent. and 2 per cent., the correction is 0.3 per cent.

Notes.—Since, as stated on page 114, the volume of the graduated portion of the neck of the bottle, from 0 to 10, is 2 cc., if 20 cc. of the extract are taken the readings will be directly in per cent. by volume.

The separated oil may be examined for its refractive index in the Abbe refractometer, if desired, but this determination has little practical value on account of the presence of alcohol in the separated oil. The alcohol makes the readings on the refractometer usually 0.002 or more lower than the correct figures for the lemon oil. The refractive index of lemon oil is 1.4750 at 20°C.

Alcohol.—In the absence of lemon oil, as indicated by the preliminary test, the alcohol may be determined as under Whiskey, page 477. If an appreciable amount of oil is present, however, it must be removed, since otherwise it would pass into the distillate. To do this, dilute 20 cc. of the extract to 100 cc. with water, and pour the mixture into a dry Erlenmeyer flask containing 5 grams of light magnesium carbonate. Shake thoroughly and filter through a dry filter. Measure out 50 cc. of the clear filtrate, add about 15 cc. of water and distil 50 cc., as directed on page 416. From the specific gravity of the distillate determine the per cent. of alcohol by volume and multiply by 5 to obtain the percentage in the original extract.

Notes.—The magnesium carbonate serves to absorb the oil precipitated by the dilution, and prevents it passing through the filter.

The distillation of the alcohol after previous extraction of the volatile oil by petroleum ether, as described under Ginger Extract, page 407, can also be used, but for the relatively in-

soluble lemon oil, the removal by magnesium carbonate is simpler and satisfactory.

If it is not required to determine the exact amount of alcohol in the extract, an approximate estimation may be made by determining the specific gravity of the extract itself without distilling. Lemon oil has a specific gravity (0.860) which corresponds to an alcohol percentage of 81.5, so that in the absence of appreciable quantities of sugar or glycerin the determination is reasonably accurate.

Methyl alcohol has occasionally been reported in lemon extracts in the past, being used in place of the more expensive grain alcohol. Stringent legislation, however, has practically put a stop to its use, so that it would be difficult to find a sample containing it today. Its presence may be shown by tests described under Alcoholic Foods, page 426.

Citral.[1] —*Reagents.*—(*a*) *Metaphenylenediamine hydrochloride Solution.*—Prepare a 1 per cent. solution of metaphenylenediamine hydrochloride in 95 per cent. alcohol. If the reagent is of good quality the solution will be bright and clear, free from suspended matter and practically colorless. If it shows much color, shake with fullers' earth and filter through a double filter. Prepare only such an amount as can be used within a few hours, as it darkens on standing.

(*b*) *Alcohol.*—The best quality of cologne spirits will be found suitable for use without further treatment. If, however, the alcohol available is at all colored, or if the metaphenylenediamine solution prepared as above shows a distinct yellow color (due to aldehydes) which is not removed by the fullers' earth, the alcohol used should be treated to remove most of the aldehyde. This can be done readily by the use of silver oxide, as mentioned on page 158.

Method.—All the operations are to be carried on at room temperature. Weigh into a 50-cc. graduated flask 25 grams of the extract and make up to the mark with alcohol. Mix thoroughly, transfer 2 cc. with a pipette to another 50-cc. flask, add 10 cc. of the metaphenylenediamine hydrochloride reagent and make up to the mark. Compare the color in a Duboscq, or other suitable colorimeter, with that given by 2 cc. of a standard citral solution

[1] Hiltner: *Bur. of Chem., Bull.* **132,** p. 102.

(1 cc. = 1 mgm. of citral) which has been treated with 10 cc. of the reagent and made up to 50 cc. with 95 per cent. alcohol in the same way. From this first determination calculate the amount of the standard citral solution which should be used in order that the color may equal approximately that of 2 cc. of the diluted sample and repeat the determination accordingly.

Notes.—The citral used in preparing the standard solution should be re-distilled in order to ensure its purity (boiling point is 228°C.) and kept cold and in the dark, best in a refrigerator. Under these conditions it should keep for six months with but little change. The dilute standard should be used the same day that it is prepared, since it loses strength.

If the extract contains a considerable amount of cane sugar the results obtained by the Hiltner method will usually be from 10 to 30 per cent. too high, and in this case it will be better to determine the citral by the fuchsin-sulphite method, described on page 478, but it should be remembered that the result will represent the total aldehydes present rather than the citral. Fuchsin-sulphurous acid being a general reagent for aldehyde, the small amount of citronella in the lemon oil is included with the citral.

Color.—Evaporate a portion of the extract to dryness on the water-bath to expel alcohol and essential oil, take up the residue in water and test for coal-tar colors by the double dyeing method described on page 60. In dyeing the color on wool care should be taken to have the bath only very faintly acid, since Naphthol Yellow S, the color most likely to be present, is decolorized by slight excess of acid. One small drop of acetic or dilute hydrochloric acid will be sufficient. If a coal-tar dye is not found, test for turmeric or lemon-peel color.

Turmeric.—Add to 25 or 50 cc. of the sample 3 drops of saturated boric acid solution, 1 small drop of dilute (1:10) hydrochloric acid, and a piece of filter-paper so arranged that it is only half immersed in the liquid. Evaporate to dryness on the water-bath. In the presence of turmeric the paper will be colored pink and the test may be confirmed as described on page 98. Excess of hydrochloric acid should be avoided as in testing for boric acid.

Lemon Peel Color.[1]—Dilute a few cubic centimeters of the extract until the color has nearly disappeared and divide the solution between two test-tubes. To one add a few drops of concentrated hydrochloric acid and to the other a few drops of strong ammonia. In the presence of the natural color of the lemon peel a distinct yellow color should result in each case.

Interpretation of Results

The results will usually require but little study in order to distinguish genuine from adulterated extracts. A pure extract will contain at least 5 per cent. of lemon oil and ordinarily 80 per cent. or more of alcohol. The amount of citral should be between 0.20 and 0.30 per cent. Factitious or imitation ex-

Table LXXI.—Analyses of Lemon Extracts

Statement on label	Alcohol, per cent.	Solids, per cent.	Lemon oil, per cent.	Citral, per cent.	Color	Conclusions of analyst
"Extract of Lemon—Artificially Colored."	86.94	0.2	6.1	0.27	Turmeric.	Genuine lemon extract.
"Pure Concentrated Flavor-Lemon."	47.2	0.07	0.9	0.04	Naphthol Yellow S.	Dilute lemon extract colored.
"Confectioners' Concentration C.X.C. Lemon– Soluble,...Six times the standard strength."	60.64	5.65	1.6	0.56	.....	Misbranded as to strength; not concentrated.
"Special Lemon Flavor (Strengthened with Citral)."	45.5	0.10	0.0	0.24	Coal-tar dye.	Imitation lemon flavor prepared from citral, alcohol and color.
"Concentrated Extract of Terpeneless Lemon."	57.0	....	0.4	0.10	Coal-tar dye.	Dilute terpeneless extract.
"Pure Food Extract Lemon (Soluble)—representing a strength of 5 per cent. as required by pure food laws."	29.3	0.04	0.0	0.05	Naphthol Yellow S.	Highly dilute terpeneless extract.
"Double Extract Lemon."...	7.6	0.8	0.0	0.02	Tartrazine.	Imitation extract containing no true lemon.
"Perfecto Terpeneless Lemon Flavor—Absolutely Pure."	35.2	....	0.0	0.035	Lemon peel.	Less than one-fifth of standard strength.

[1] Albrech: *Bur. of Chem., Bull.* **137**, p. 71.

tracts usually contain much less alcohol, 20 to 50 per cent., and may show in oil from nearly 5 per cent. to none at all. The citral content may be very low (0.02 to 0.05 per cent.) in the weak extracts, or distinctly higher than normal in extracts which have been re-inforced with citral.

Table LXXI illustrates typical analyses, taken mainly from the Notices of Judgment issued under the Federal Food and Drugs Act.

Extract of Ginger

Ginger extract is defined[1] as "the flavoring extract prepared from ginger and contains in each one hundred (100) cubic centimeters, the alcohol-soluble matters from not less than twenty (20) grams of ginger." The product is practically the same as the "tincture of ginger" of the U. S. Pharmacopœia, the only apparent difference being in the fact that the U. S. P. preparation is required to be made with 95 per cent. alcohol, while the definition of the "extract" does not specify the alcoholic strength. It can be readily shown, however, that unless strong alcohol of at least 70 per cent. be used, the "alcohol-soluble matters" of the ginger will not be entirely extracted.

The process of the Pharmacopœia for 200 grams of ginger is as follows:

> Moisten the ginger with 60 cc. of alcohol, transfer to a percolator and allow it to stand for 6 hours; pack firmly and pour on enough alcohol to saturate the powder. When the liquid begins to drop from the percolator, close the lower orifice, and macerate, covered closely, for 24 hours. Then allow the percolation to proceed slowly, pouring on sufficient alcohol to obtain 1 liter of the tincture.

By this extraction of the powdered ginger with strong alcohol there is obtained a dark colored aromatic liquid with the characteristic flavor of ginger and containing essentially all of the pungent principles of the root. The extract may also be prepared more simply by dissolving the "oleoresin" of ginger in strong alcohol.

This oleoresin, of which ginger contains approximately 5 per cent., is practically the resin, which is of a phenolic nature, dissolved in the essential oil. The pungency of ginger is supposed

[1] *U. S. Dept. of Agr., Office of the Secretary, Circular* **19.**

to be due to the resin while the aroma is caused by the essential oil. It may be extracted from the ginger by means of acetone, petroleum ether or other suitable solvent, which can be distilled off at low temperature, leaving the oleoresin as a viscous liquid. The extract prepared from the oleoresin, if 95 per cent. alcohol is used, is almost identical with the U. S. P. tincture.

The exact composition of the extract is dependent to a certain extent upon the kind of ginger employed, Jamaica ginger, which is considered most desirable, not containing on the average so large a proportion of alcohol-soluble matter as some other varieties. As will be shown later, the composition varies very considerably with the strength of alcohol employed for extraction, especially if concentrations below 70 per cent. by volume are employed.

Forms of Adulteration.—The most common form of adulteration, as in the case of lemon extract, results primarily from economizing in the cost of manufacture by the employment of weak alcohol. In this respect ginger extract is unique, since weak alcohol extracts considerably more than does strong alcohol, the water-soluble material of the root being greatly in excess of the alcohol-soluble. The value of the extract, however, is not to be measured by the total material taken from the ginger by the solvent, but rather by the alcohol-soluble portion, since this will contain the valuable oil and oleoresin, which are insoluble in water. The quantity of dissolved matter present is, moreover, no criterion of the quality of the extract for the reason that sugar, molasses, glycerin or similar substances are added to change the flavor of the extract or to give it more "body."

Weak extracts may be helped out in appearance by the use of caramel or other colors and often owe their pungency to the addition of capsicum or extract of cayenne. In some cases ginger may be almost entirely absent, a trace of capsicum in the dilute extract simulating the true ginger.

Analytical Methods

Specific Gravity.—Determine at 15.5°C. as described under General Methods, page 3.

Alcohol.—The ordinary methods for determining alcohol (see page 416) are not suitable on account of the volatile essential oil. The method used in the case of lemon extract, page 401, can be employed, but if exact results are desired the following method[1] is better:

Dilute 25 cc. of the extract to about 100 cc., place in a separatory funnel, and add finely powdered salt until saturated. Shake thoroughly for 5 minutes with 50 cc. light petroleum ether (boiling below 60°C.), let stand for half an hour and draw off the lower layer. Wash the petroleum ether twice successively with 25 cc. of saturated salt solution and add to the first aqueous solution, giving a total volume of about 150 cc. Distil 100 cc. and determine the alcohol in the distillate as on page 416. Multiply by 4 to obtain the alcohol in the original sample.

Notes.—The method is a more general one than that described under lemon extracts, being applicable to alcoholic solutions of essential oils which are not precipitated quantitatively by adding water and hence cannot be removed by magnesium carbonate. It can also be employed for the determination of alcohol in liquids containing such volatile compounds as chloroform, ether and compound esters, by making two extractions with petroleum ether.

The volatile ginger oil, which would otherwise pass into the distillate with the alcohol, is readily extracted by the petroleum ether especially when "salted out" by the addition of the sodium chloride, its solubility being thereby considerably decreased. The method is a familiar one in many operations of organic chemistry.

Total Solids.—Weigh 10 grams into a flat-bottom platinum dish and evaporate on the copper top (not over the live steam) of the water-bath. Dry to constant weight in the drying oven at the temperature of boiling water. Carry out the determination in duplicate.

Solids Soluble in Alcohol.—Add 15 cc. of 95 per cent. alcohol to one of the dry residues obtained in the previous determination. Stir thoroughly with a glass rod and allow to stand for an hour. Wash into a 50-cc. flask with 95 per cent. alcohol and

[1] Thorpe and Holmes: *J. Chem. Soc.*, **1903**, 314.

make up to the mark. Filter through a dry fluted filter, evaporate 25 cc. and weigh as in total solids, taking the same precautions to evaporate the alcohol slowly.

Solids Soluble in Water.—Add 15 to 20 cc. of water at room temperature to the other residue obtained in the total solids determination. Stir with a glass rod during 3 hours, taking especial pains to secure intimate mixing with the solvent, which is sometimes difficult on account of the gummy character of the residue. Wash into a 50-cc. flask with water and make up to the mark. Filter through a dry fluted filter, evaporate 25 cc. of the filtrate and dry to constant weight in a water-oven.

Detection of Ginger.[1]—Dilute 10 c.c. of the extract to 30 cc., evaporate to 20 cc. to remove the alcohol, transfer to a separatory funnel and extract with an equal volume of ether. Evaporate the ether spontaneously in a porcelain dish. Add to the residue 10 or 12 drops of concentrated sulphuric acid and about 5 mg. of vanillin, mix thoroughly with a glass rod; allow a few drops of water to flow down the sides of the dish and touch the edge of the acid mixture. A persistent dark blue color indicates ginger.

Detection of Capsicum.[2]—To 10 cc. of the extract add cautiously dilute sodium hydroxide until the solution is very slightly alkaline to litmus paper. Evaporate at about 70°C. to about one-quarter of the original volume and make slightly acid with dilute sulphuric acid, testing with litmus paper. Transfer to a separatory funnel, rinsing the evaporating dish with water, and extract with an equal volume of ether. Avoid emulsification, shaking the funnel gently for a minute or two. Draw off the lower layer and wash the ether extract once with 10 cc. of water. Transfer the washed ether extract to a small evaporating dish, make decidedly alkaline with half-normal alcoholic potash, and evaporate at about 70°C. until the residue is pasty; then add about 20 cc. more of half-normal alcoholic potash and allow to stand on the water-bath for half an hour, or until the gingerol is completely saponified. Dissolve the residue in a little water and transfer with water to a small separatory funnel. The volume should not

[1] Seeker: *Bur. of Chem., Bull.* **137,** p. 75; Mitchell: *Bur. of Chem., Bull.* **152,** p. 137.

[2] Garnett and Green: *Brit. and Col. Druggist,* **1907;** LaWall: *Am. J. Pharm.,* **1909,** 218; Doyle: *Bur. of Chem., Bull.* **152,** p. 145.

exceed 50 cc. Extract the alkaline solution with an equal volume of ether and wash the ether until neutral to litmus. Transfer the washed ether to a small evaporating dish and allow it to evaporate spontaneously. Finally, test the residue for capsicum by moistening the tip of the finger, rubbing it around on the bottom and sides of the dish and then applying the finger to the end of the tongue. A hot, stinging or prickly sensation, which persists for several minutes, indicates capsicum.

Notes.—The test depends upon the fact that gingerol, the resin of ginger, to which its pungency is due, is saponified and decomposed by heating with alkali, while capsaicin, the active principle of capsicum, remains unaffected. The addition of dilute alkali at first is to prevent the loss of capsaicin by volatilization during the evaporation.

The test is a striking illustration of the great delicacy of some physiological tests as compared with ordinary chemical reactions. There is no color reaction, even, known for capsaicin which will detect the presence of less than 1 part in 1000, while a distinct "bite" is produced on the tongue by 1 part in 1,000,000.

Detection of Caramel.[1]—The coloring matter of pure ginger extracts is completely soluble in amyl alcohol acidified with phosphoric acid (Marsh reagent) while caramel is insoluble. Hence, carry out the test as described on page 388 and note the resulting colors in the two separated layers of liquid.[2] If the lower or aqueous layer is colorless, caramel is absent. If caramel be present, the lower layer will be colored yellowish brown, the intensity of color being proportional to the amount of caramel present. By comparing the color of the original sample with that of the portion extracted by the Marsh reagent, the approximate amount of added color may be determined.

Interpretation of Results

Since the standard is based on the presence in the extract of the "alcohol-soluble matters" from a certain proportion of ginger,

[1] Hiltner: *Bur. of Chem., Bull.* **162,** p. 91.

[2] It may be necessary if a precipitate forms on diluting the extract on making to volume with 50 per cent. alcohol, to compare the color of the aqueous layer with the color of the original extract, rather than with the untreated portion of the diluted extract as directed in Hiltner's test.

it is first essential to determine the quantity of such material that would be found in an extract properly prepared. Street and Morison[1] give the following analysis of two samples of U. S. P. ginger tincture prepared in the laboratory:

Variety	Sp. gr. (15.6°C.)	Alcohol, per cent.	Total solids, per cent.	Alcohol-soluble solids, per cent.	Water-soluble solids, per cent.
Jamaica	0.8198	94.63	1.43	1.42	0.21
African	0.8222	93.21	1.81	1.81	0.16

Similar values were found by Lythgoe and Nurenberg[2] for genuine ginger tinctures (Table LXXII).

TABLE LXXII.—COMPOSITION OF GENUINE GINGER EXTRACT

Variety	Sp. gr. 20°/4°	Alcohol, per cent.	Total solids, per cent.	Alcohol-soluble solids, per cent.	Water-soluble solids, per cent.	n_D^{20}
Jamaica	0.8184	89.76	1.40	1.33	0.24	1.3662
Jamaica	0.8174	89.24	1.38	1.10	0.19	1.3658
Jamaica	0.8189	88.84	1.60	1.24	0.22	1.3657
African	0.8173	89.36	1.86	1.84	0.08	1.3665
Cochin	0.8181	89.24	2.19	1.94	0.22	1.3666
Ginger oleoresin	0.8144	91.12	1.24	1.16	0.10	1.3663
Average	0.8180	89.29	1.68	1.49	0.19	1.3662

As pointed out on page 406, the determination of total solids alone is not evidence that the extract contains the alcohol-soluble matters of ginger. Alcohol of less strength means a higher percentage of solids in the extract and a greater relative proportion of water-soluble solids. The figures below show the results obtained by decreasing the strength of alcohol used for extraction:

Determination	95 per cent. alcohol	60 per cent. alcohol	20 per cent. alcohol
Total solids	1.43	1.91	2.50
Alcohol-soluble solids	1.42	1.16	0.30
Water-soluble solids	0.21	1.23	2.09

[1] *Bur. of Chem., Bull.* **137**, p. 76.
[2] *J. Ind. Eng. Chem.*, **1911**, 910.

Any concentration of alcohol above 70 per cent., however, gives practically the same results as the 95 per cent.

Street and Morison suggest that a properly prepared ginger extract should have a specific gravity of about 0.820, and should contain at least 93 per cent. of alcohol by volume and 1 to 2 per cent. of solids, practically all of which should be soluble in 95 per cent. alcohol, and not over 15 per cent. soluble in cold water. In the examination of commercial extracts, however, which are not necessarily prepared by the Pharmacopœia method, the requirement as to the water-soluble solids should not be interpreted too rigidly. Fig. 57 gives the relation between alcohol

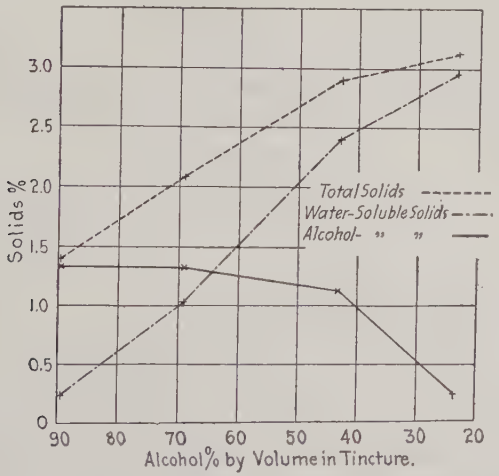


FIG. 57.—Relation of alcohol to solids in tincture of ginger.

and solids in ginger extract and shows that the alcohol-soluble solids remain practically the same as the percentage of alcohol decreases, until below 70 per cent., while the water-soluble solids and consequently the total solids gradually increase.[1] Hence a sample might show a considerable proportion of water-soluble solids and still contain in 100 cc. the specified quantity of "alcohol-soluble matters." The percentage of alcohol-soluble solids is therefore a better index of the quality of the extract and should conform reasonably closely to the values found in the analyses of genuine extracts.

[1] Lythgoe and Nurenberg: *Loc. cit.*

Harrison and Sullivan[1] point out that some of the adulterants of ginger extracts, as molasses, caramel, glycerin or sugar, are more soluble in alcohol than in ether, hence the percentage of solids soluble in ether conveys more exact information as to the strength of a commercial ginger extract than does the amount of alcohol-soluble solids.

To determine the *ether-soluble solids*, 10 cc. are evaporated in a porcelain dish to complete dryness. Absolute ether is then added to the residue, the dish covered with a watch-glass and allowed to stand 15 minutes. The ether is then decanted through a dry filter into a tared 100-cc. Erlenmeyer flask, and the ether washing repeated. The undissolved solids remaining in the dish are scraped from the sides with a spatula and rubbed up with successive small portions of ether which are passed through the filter until no more material is dissolved, as shown by the ether coming through colorless. The ether is finally distilled off and the flask dried at 100°C. to constant weight.

TABLE LXXIII.—ANALYSES OF COMMERCIAL GINGER EXTRACTS

Sp. gr. 15.6°	Alcohol, per cent. by volume	Total solids, per cent.	Alcohol-soluble solids, per cent.	Water-soluble solids, per cent.	Remarks
0.8366	96.25	1.85	1.72	0.39	Maximum of 7 standard extracts.
0.8218	90.59	0.94	0.94	0.08	Minimum of 7 standard extracts.
0.8291	93.78	1.38	1.33	0.28	Average of 7 standard extracts.
0.8332	95.07	1.68	1.58	0.47	Maximum of 12 standard tinctures.
0.8224	90.52	1.00	1.00	0.12	Minimum of 12 standard tinctures.
0.8271	93.33	1.36	1.30	0.23	Average of 12 standard tinctures.
0.9184	59.88	2.17	1.12	1.51	Weak alcohol extract.
0.9567	54.28	9.90	1.39	9.12	Contains sugar and water.
0.9948	38.07	10.14	0.86	9.72	Contains molasses and water.
0.9588	37.28	0.49	0.42	0.41	Contains capsicum and water.
0.9557	39.35	1.18	0.54	1.01	Contains oleoresin ginger, oleoresin capsicum, essence oil ginger, caramel and water.
0.9054	54.65	1.42	0.67	1.12	Made from exhausted ginger.
0.9308	46.91	0.73	0.46	0.54	Made from oleoresin of ginger and weak alcohol.
0.9464	39.52	0.46	0.33	0.29	
0.9864	9.64	0.73	0.16	0.71	Made from oleoresin of ginger, capsicum and very dilute alcohol.
0.9960	2.10	0.43	0.28	0.39	

[1] Paper presented at 1914 meeting of A. O. A. C.

In a series of extracts prepared from several varieties of ginger, using 95 per cent. alcohol, the ratio of alcohol-soluble to total solids varied from 1 : 1.01 to 1 : 1.09; and the ether-soluble to total solids from 1 : 1.2 to 1 : 1.14. In extracts prepared with 50 per cent. alcohol, however, the ratio of alcohol-soluble to total solids was from 1 : 1.51 to 1: 7.00, with a general average of about 1: 2.5. In the case of the ether-soluble solids, however, a higher ratio was found, ranging from 1: 3.98 to 1 : 10.16, being generally above 1: 5.

In Table LXXIII are given some typical analyses of commercial extracts taken from the papers quoted above, including both standard and adulterated samples.

Selected References

GILDEMEISTER AND HOFFMANN (Trans. by Kremers).—The Volatile Oils.

HORTVET AND WEST.—Determination of Essential Oils in Flavoring Extracts. *J. Ind. Eng. Chem.*, 1909.

PARRY.—The Chemistry of Essential Oils.

WINTON AND BERRY.—Composition of Authentic Vanilla Extracts. U. S. Dept. of Agr., *Bur. of Chem., Bull.* 152.

CHAPTER XI

ALCOHOLIC FOODS

If the term food be broadened to include also beverages, then the alcoholic foods rank in analytical importance and interest with the saccharine foods and the fats and oils. It is indeed no severe tax on the imagination to regard the alcoholic beverages as essentially saccharine foods since alcohol, their chief constituent, is derived by the fermentation of the sugar either naturally present or added. Further, many of them contain notable quantities of sugars still unfermented.

The alcoholic foods group themselves naturally into two great divisions: (*a*) those like wine or beer in which the fermented product is consumed directly; and (*b*) such products as whiskey in which, for the concentration of the alcohol and modification of the flavor, the fermented product is further subjected to distillation before being consumed.

As with saccharine products and with oils, certain factors common to all will be considered first and then a typical example from each class will be discussed in greater detail.

Alcohol.—By the word *alcohol* without qualification is usually meant ethyl alcohol, a colorless, mobile liquid of characteristic odor and burning taste. It is miscible with water in all proportions, the mixing being accompanied by the evolution of heat and a distinct contraction in volume. It has a boiling point of 78.4°C. and a specific gravity of 0.79389 at $\frac{15.56°}{15.56°}$C., as adopted in this country by the Bureau of Standards, based on the work of Mendelejeff. The tables of Tralles, as employed in the Bureau of Internal Revenue, and those of the Association of Official Agricultural Chemists are based upon somewhat different values for the specific gravity of absolute alcohol and give slightly different results, although if used under the conditions for which they were standardized the results are not essentially different for relatively small amounts of alcohol.

Detection of Alcohol.—Qualitative tests for ethyl alcohol are not often required in food analysis, since ordinarily its presence is assumed in certain classes of food materials and the quantitative determination can be carried out with ease and rapidity. If, however, such a test is necessary either or both of those described below will be found satisfactory.

Occasionally the test may be applied directly to the original liquid, but in most cases it will be found better to concentrate it by distillation. This may be done by direct distillation after the addition of salt, by which the alcohol is distilled freer from water, or better by distilling the neutralized liquid through a simple fractionating tower, such as the ordinary Glinsky tube (Fig. 58).

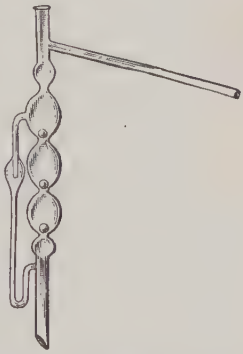


Fig. 58.—Glinsky fractionating tube.

(*a*) *Iodoform Test.*—To 5 cc. of the first portion of the distillate obtained above add 10 drops of 10 per cent. sodium hydroxide, then add from a medicine dropper a solution of iodine in potassium iodide until a very slight permanent yellow color is produced. Let the solution stand several minutes, then shake and note whether any iodoform has separated. If no iodoform separates in the cold, immerse the bulb of a small thermometer in the solution, heat to 60°C. for a minute and set aside. The presence of alcohol is shown by the gradual formation of a yellowish crystalline precipitate of iodoform, CHI_3, with characteristic odor.

Notes.—Acetone gives the reaction in the cold, ethyl alcohol and several other substances quite readily at 60°, hence a positive test should not be accepted as conclusive evidence of the presence of alcohol, but should be confirmed by the benzoyl chloride test (below) or by the specific gravity or refractive index of the distillate.

An odor of iodoform, unaccompanied by a perceptible precipitate, should not be regarded as a positive test.

(*b*) *Ethyl Benzoate Test.*—To a small portion of the distillate obtained as described above add a few drops of benzoyl chloride and several cc. of 10 per cent. sodium hydroxide solution. Shake

and warm gently. In the presence of ethyl alcohol the ethyl benzoic ester is formed according to the following reaction:

$$C_2H_5OH + C_6H_5COOH \rightarrow C_2H_5O - COC_6H_5 + H_2O$$

and may be identified by its characteristic odor, and if formed in sufficient quantity by its boiling point (212°C.).

Note.—If a positive result is obtained in the two tests outlined, it may be assumed with reasonable certainty that ethyl alcohol is present, since the only benzoic ester whose odor might be confused with that of the ethyl ester is the methyl ester, and methyl alcohol does not give the iodoform reaction. If further evidence is required, it would best be secured from the specific gravity or refractive index of the distillate as described below under the quantitative estimation of alcohol.

Determination of Alcohol.—Measure or weigh (see Notes, page 425) a suitable quantity of the sample into a 500 cc. round-bottomed flask and dilute to 150 cc. With liquids containing more than 25 per cent. of alcohol, as distilled liquors or extracts, use 25 cc.; with liquids containing a less percentage of alcohol, as wines, use 100 cc. With some wines or other samples which are distinctly acid, the acidity should be neutralized with dilute sodium hydroxide before distilling, or a pinch (0.1–0.2 gram) of precipitated calcium carbonate may be added to the flask. Distil, rather slowly at first, about 95 cc. into a 100 cc. graduated flask with a narrow neck, so arranged that the tip of the condenser is inserted some little distance into the neck of the flask. Fill to the mark (at the proper temperature) with distilled water, mix thoroughly and take the specific gravity with a pyknometer, preferably at either 15.56° or 20°C. Note the precautions detailed under General Methods, pages 2 to 4.

Calculation of the Result.—If the per cent. of alcohol by volume is desired and the weighings of the pyknometer filled with the distillate and with water were made at 15.56°, the value may be taken directly from Table LXXVI and converted to per cent. by weight by means of Table LXXVIII. If, on the other hand, the determination were made at 20° or 25°, which is a more desirable temperature, the specific gravity should be multiplied by the density of water at 20° (or 25°) taken from Table I, page 6, in order to obtain the density at $\frac{20°}{4°}$ or $\frac{25°}{4°}$. By interpolation, in Table

TABLE LXXIV.—DENSITY[1] OF MIXTURES OF ETHYL ALCOHOL AND WATER

Per cent. alcohol by weight	$D\frac{15}{4}$	$D\frac{20}{4}$	$D\frac{25}{4}$	Per cent. alcohol by weight	$D\frac{15}{4}$	$D\frac{20}{4}$	$D\frac{25}{4}$
0	0.99913	0.99823	0.99708	50	0.91776	0.91384	0.90985
1	725	636	520	51	555	160	760
2	542	453	336	52	333	.90936	534
3	365	275	157	53	110	711	307
4	195	103	.98984	54	.90885	485	079
5	032	.98938	817	55	659	258	.89850
6	.98877	780	656	56	433	031	621
7	729	627	500	57	207	.89803	392
8	584	478	346	58	.89980	574	162
9	442	331	193	59	752	344	.88931
10	304	187	043	60	523	113	699
11	171	047	.97897	61	293	.88882	466
12	041	.97910	753	62	062	650	233
13	.97914	775	611	63	.88830	417	.87998
14	790	643	472	64	597	183	763
15	669	514	334	65	364	.87948	527
16	552	387	199	66	130	713	291
17	433	259	062	67	.87895	477	054
18	313	129	.96923	68	660	241	.86817
19	191	.96997	782	69	424	004	579
20	068	864	639	70	187	.86766	340
21	.96944	729	495	71	.86949	527	100
22	818	592	348	72	710	287	.85859
23	689	453	199	73	470	047	618
24	558	312	048	74	229	.85806	376
25	424	168	.95895	75	.85988	564	134
26	287	020	738	76	747	322	.84891
27	144	.95867	576	77	505	079	647
28	.95996	710	410	78	262	.84835	403
29	844	548	241	79	018	590	158
30	686	382	067	80	.84772	344	.83911
31	524	212	.94890	81	525	096	664
32	357	038	709	82	277	.83848	415
33	186	.94860	525	83	028	599	164
34	011	679	337	84	.83777	348	.82913
35	.94832	494	146	85	525	095	660
36	650	306	.93952	86	271	.82840	405
37	464	114	756	87	014	583	148
38	273	.93919	556	88	.82754	323	.81888
39	079	720	353	89	492	062	626
40	.93882	518	148	90	227	.81797	362
41	682	314	.92940	91	.81959	529	094
42	478	107	729	92	688	257	.80823
43	271	.92897	516	93	413	.80983	549
44	062	685	301	94	134	705	272
45	.92852	472	085	95	.80852	424	.79991
46	640	257	.91868	96	566	138	706
47	426	041	649	97	274	.79846	415
48	211	.91823	429	98	.79975	547	117
49	.91995	604	208	99	670	243	.78814
50	776	384	.90985	100	360	.78934	506

[1] $D\frac{15}{4}$ = Density at 15°C. referred to water at 4°C.

TABLE LXXV.—DENSITY OF MIXTURES OF ETHYL ALCOHOL AND WATER AT $\frac{20°}{4°}$ C.

Per cent. alcohol by weight	Tenths of per cent.									
	0	1	2	3	4	5	6	7	8	9
0	0.99823	804	785	766	748	729	710	692	673	655
1	636	618	599	581	562	544	525	507	489	471
2	453	435	417	399	381	363	345	327	310	292
3	275	257	240	222	205	188	171	154	137	120
4	103	087	070	053	037	020	003	*987	*971	*954
5	.98938	922	906	890	874	859	843	827	811	796
6	780	765	749	734	718	703	688	673	658	642
7	627	612	597	582	567	553	538	523	508	493
8	478	463	449	434	419	404	389	374	360	345
9	331	316	301	287	273	258	244	229	215	201
10	187	172	158	144	130	117	103	089	075	061
11	047	033	019	006	*992	*978	*964	*951	*937	*923
12	.97910	896	883	869	855	842	828	815	801	788
13	775	761	748	735	722	709	696	683	670	657
14	643	630	617	604	591	578	565	552	539	526
15	514	501	488	475	462	450	438	425	412	400
16	387	374	361	349	336	323	310	297	284	272
17	259	246	233	220	207	194	181	168	155	142
18	129	116	103	089	076	063	050	037	024	010
19	.96997	984	971	957	944	931	917	904	891	877
20	864	850	837	823	810	796	783	769	756	742
21	729	716	702	688	675	661	647	634	620	606
22	592	578	564	551	537	523	509	495	481	467
23	453	439	425	411	396	382	368	354	340	326
24	312	297	283	269	254	240	225	211	196	182
25	168	153	139	124	109	094	080	065	050	035
26	020	005	*990	*975	*959	*944	*929	*914	*898	*883
27	.95867	851	836	820	805	789	773	757	742	726
28	710	694	678	662	646	630	613	597	581	565
29	548	532	516	499	483	466	450	433	416	400
30	382	365	349	332	315	298	281	264	247	230
31	212	195	178	161	143	126	108	091	074	056
32	038	020	003	*985	*967	*950	*932	*914	*896	*878
33	.94860	842	824	806	788	770	752	734	715	697
34	679	660	642	624	605	587	568	550	531	512
35	494	475	456	438	419	400	382	363	344	325
36	306	287	268	249	230	211	192	172	153	134
37	114	095	075	056	036	017	*997	*978	*958	*939
38	.93919	899	879	859	840	820	800	780	760	740
39	720	700	680	660	640	620	599	579	559	539
40	518	498	478	458	437	417	396	376	356	335
41	314	294	273	253	232	212	191	170	149	129
42	107	086	065	044	023	002	*981	*960	*939	*918
43	.92897	876	855	834	812	791	770	749	728	707
44	685	664	642	621	600	579	557	536	515	493
45	472	450	429	408	386	365	343	322	300	279
46	257	236	214	193	171	150	128	106	085	063
47	041	019	*997	*976	*954	*932	*910	*889	*867	*845
48	.91823	801	780	758	736	714	692	670	648	626
49	604	582	560	538	516	494	472	450	428	406
50	384	361	339	317	295	272	250	228	206	183

* The asterisk indicates a diminution of one in the second place decimal.

TABLE LXXV.—DENSITY OF MIXTURES OF ETHYL ALCOHOL AND WATER AT $\frac{20°}{4°}$C.—(*Continued*)

Per cent. alcohol by weight	Tenths of per cent.									
	0	1	2	3	4	5	6	7	8	9
50	0.91384	361	339	317	295	272	250	228	206	183
51	160	138	116	093	071	049	026	004	*981	*959
52	.90936	914	891	869	846	824	801	779	756	734
53	711	689	666	644	621	598	576	553	531	508
54	485	463	440	417	395	372	349	327	304	281
55	258	236	213	190	167	145	122	099	076	054
56	031	008	*985	*962	*939	*917	*894	*871	*848	*825
57	.89803	780	757	734	711	688	665	643	620	597
58	574	551	528	505	482	459	436	413	390	367
59	344	321	298	275	252	229	206	183	160	137
60	113	090	067	044	021	*998	*975	*951	*928	*905
61	.88882	859	836	812	789	766	743	720	696	673
62	650	626	603	580	557	533	510	487	463	440
63	417	393	370	347	323	300	277	253	230	206
64	183	160	136	113	089	066	042	019	*995	*972
65	.87948	925	901	878	854	831	807	784	760	737
66	713	689	666	642	619	595	572	548	524	501
67	477	454	430	406	383	359	336	312	288	265
68	241	218	194	170	147	123	099	075	052	028
69	004	*981	*957	*933	*909	*885	*862	*838	*814	*790
70	.86766	742	718	694	671	647	623	599	575	551
71	527	503	479	455	431	407	383	359	335	311
72	287	263	239	215	191	167	143	119	095	071
73	047	022	*998	*974	*950	*926	*902	*878	*854	*830
74	.85806	781	757	733	709	685	661	636	612	588
75	564	540	515	491	467	443	419	394	370	346
76	322	297	273	249	225	200	176	152	128	103
77	079	055	031	006	*982	*958	*933	*909	*884	*860
78	.84835	811	787	762	738	713	689	664	640	615
79	590	566	541	517	492	467	443	418	393	369
80	344	319	294	270	245	220	196	171	146	121
81	096	072	047	022	*997	*972	*947	*923	*898	*873
82	.88848	823	798	773	748	723	698	674	649	624
83	599	574	549	523	498	473	448	423	398	373
84	348	323	297	272	247	222	196	171	146	120
85	095	070	044	019	*994	*968	*943	*917	*892	*866
86	.82840	815	789	763	738	712	686	660	635	609
87	583	557	531	505	479	453	427	401	375	349
88	323	297	271	245	219	193	167	140	114	088
89	062	035	009	*983	*956	*930	*903	*877	*850	*824
90	.81797	770	744	717	690	664	637	610	583	556
91	529	502	475	448	421	394	366	339	312	285
92	257	230	203	175	148	120	093	066	038	010
93	.80983	955	928	900	872	844	817	789	761	733
94	705	677	649	621	593	565	537	509	480	452
95	424	395	367	338	310	281	253	224	195	166
96	138	109	080	051	022	*993	*963	*934	*905	*875
97	.79846	816	787	757	727	698	668	638	608	578
98	547	517	487	456	426	396	365	335	305	274
99	243	213	182	151	120	089	059	028	*997	*966
100	.78934									

* The asterisk indicates a diminution of one in the second place decimal.

TABLE LXXVI.—SPECIFIC GRAVITY AT $\frac{60°}{60°}$F. $\left(\frac{15.56°}{15.56°}\text{C.}\right)$ OF MIXTURES (BY VOLUME) OF ETHYL ALCOHOL AND WATER

Per cent. alcohol by volume at 60°F.	Tenths of per cent.									
	0	1	2	3	4	5	6	7	8	9
0	1.00000	*985	*970	*955	*940	*925	*910	*895	*880	*865
1	.99850	835	820	806	791	776	761	747	732	717
2	703	688	674	659	645	630	616	602	587	573
3	559	545	531	516	502	488	474	460	446	432
4	419	405	391	378	364	350	336	323	309	296
5	282	269	255	242	228	215	202	189	176	163
6	150	137	124	111	098	085	073	060	047	035
7	022	009	*997	*984	*972	*960	*947	*935	*923	*911
8	.98899	887	875	863	851	838	826	814	803	791
9	779	767	755	743	731	720	708	696	684	672
10	661	649	637	625	614	602	590	579	567	556
11	544	532	521	509	498	487	475	464	452	441
12	430	419	408	396	385	374	363	352	341	330
13	319	308	297	286	275	264	254	243	232	221
14	210	200	190	179	168	157	147	136	125	115
15	104	093	083	072	062	051	040	030	019	009
16	.97998	988	977	967	956	946	936	925	915	905
17	895	885	875	864	854	844	834	824	814	804
18	794	784	774	764	754	744	734	724	714	704
19	694	684	674	664	654	645	635	625	615	605
20	596	586	576	566	556	546	536	526	516	506
21	496	486	476	466	456	446	436	425	415	405
22	395	385	375	365	354	344	334	324	313	303
23	293	283	272	262	252	241	231	221	210	200
24	189	179	168	158	147	137	126	116	105	095
25	084	073	063	052	042	031	020	010	*999	*988
26	.96978	967	957	946	935	924	914	903	892	881
27	870	859	848	837	826	815	804	793	782	771
28	760	749	738	727	715	704	693	682	671	659
29	648	637	625	614	603	591	580	568	557	546
30	534	522	511	499	488	476	464	453	441	429
31	418	406	394	382	370	358	346	334	321	309
32	296	284	271	259	246	234	221	209	196	183
33	170	157	144	132	119	105	093	080	067	054
34	041	028	015	002	*988	*975	*962	*948	*935	*921
35	.95908	894	881	867	854	840	826	812	798	784
36	770	756	742	728	714	700	685	671	657	643
37	628	614	599	585	570	556	541	526	512	497
38	482	467	452	437	423	408	393	378	362	347
39	332	317	302	286	271	256	240	225	209	194
40	178	162	147	131	115	100	084	068	052	036
41	020	004	*988	*972	*956	*940	*923	*907	*891	*875
42	.94858	842	825	809	792	776	759	743	726	710
43	693	676	660	643	626	609	592	575	558	541
44	524	507	490	473	455	438	421	403	386	369
45	351	334	316	298	281	263	245	228	210	192
46	174	156	138	120	102	084	066	048	030	011
47	93993	975	956	938	920	901	883	864	845	827
48	808	789	771	752	733	714	695	676	657	638
49	619	600	581	562	543	523	504	485	465	446
50	426	407	387	368	348	328	309	289	270	250

* The asterisk indicates a diminution of one in the second place decimal.

TABLE LXXVI.—SPECIFIC GRAVITY AT $\frac{60°}{60°}$F. $\left(\frac{15.56°}{15.56°}\text{C.}\right)$ OF MIXTURES (BY VOLUME) OF ETHYL ALCOHOL AND WATER.—(*Continued*)

Per cent. alcohol by volume at 60°F.	Tenths of per cent.									
	0	1	2	3	4	5	6	7	8	9
50	.93426	407	387	368	348	328	309	289	270	250
51	230	210	190	171	151	131	111	091	071	051
52	031	011	*991	*971	*951	*931	*911	*890	*870	*850
53	.92830	810	789	769	749	728	708	688	667	647
54	626	605	585	564	544	523	502	482	461	440
55	419	398	377	357	336	315	294	273	252	231
56	210	189	168	147	126	105	084	062	041	020
57	.91999	978	956	935	914	892	871	849	827	806
58	784	762	741	719	697	675	653	631	610	588
59	565	543	521	499	477	455	433	410	388	366
60	344	322	299	277	255	232	210	188	165	143
61	120	097	075	052	030	007	*984	*962	*939	*916
62	.90893	870	847	825	802	779	756	733	710	687
63	664	641	618	595	572	549	526	503	480	457
64	434	411	388	365	341	318	295	272	249	225
65	202	179	155	132	108	085	061	038	014	*991
66	.89967	943	920	896	872	848	825	801	777	753
67	729	705	681	657	633	609	585	561	537	513
68	489	465	441	416	392	368	343	319	295	270
69	245	220	196	171	147	122	098	073	048	024
70	.88999	974	950	925	900	875	850	825	801	776
71	751	725	700	675	650	625	600	574	549	524
72	499	474	448	423	397	372	346	321	296	270
73	244	218	193	167	141	116	090	064	039	013
74	.87987	961	935	910	884	858	832	806	780	754
75	728	702	676	650	623	597	571	545	518	492
76	465	439	412	386	359	332	306	279	252	226
77	199	172	145	118	092	065	038	011	*984	*957
78	.86929	902	875	847	820	793	766	738	711	684
79	656	629	601	574	546	518	491	463	435	408
80	380	352	324	296	269	241	213	185	157	129
81	100	072	044	015	*987	*959	*931	*902	*874	*846
82	.85817	789	760	732	703	674	646	617	588	560
83	531	502	473	444	415	386	357	328	299	270
84	240	211	181	152	122	093	063	033	004	*974
85	.84944	914	884	854	824	794	764	734	703	673
86	642	612	581	551	520	490	459	428	398	367
87	336	305	274	243	212	181	150	119	088	056
88	025	*994	*962	*930	*899	*867	*835	*803	*771	*739
89	.83707	675	643	610	578	545	513	480	447	415
90	382	349	315	282	249	216	183	150	116	083
91	049	015	*981	*947	*913	*879	*845	*810	*776	*741
92	.82705	670	635	600	565	529	494	458	423	387
93	351	315	279	243	206	170	133	096	059	022
94	.81984	947	909	871	834	796	757	719	681	642
95	603	564	525	486	446	407	367	327	287	247
96	206	165	125	084	042	001	*960	*918	*876	*834
97	.80792	750	707	664	620	577	533	489	445	401
98	356	311	265	219	173	127	080	033	*985	*937
99	.79889	841	792	743	693	643	593	543	492	441
100	389									

* The asterisk indicates a diminution of one in the second place decimal.

TABLE LXXVII.—PERCENTAGES BY VOLUME AT 60°F., CORRESPONDING TO VARIOUS PERCENTAGES BY WEIGHT IN MIXTURES OF ETHYL ALCOHOL AND WATER

Per cent. alcohol by weight	Tenths of per cent.									
	0	1	2	3	4	5	6	7	8	9
0	0.000	0.126	0.252	0.378	0.504	0.630	0.755	0.881	1.007	1.132
1	1.257	1.382	1.508	1.633	1.759	1.884	2.009	2.134	2.260	2.385
2	2.510	2.635	2.760	2.885	3.010	3.135	3.259	3.384	3.509	3.633
3	3.758	3.883	4.007	4.132	4.256	4.381	4.505	4.629	4.754	4.878
4	5.002	5.126	5.250	5.374	5.499	5.623	5.747	5.871	5.995	6.119
5	6.243	6.367	6.491	6.614	6.738	6.862	6.985	7.109	7.232	7.356
6	7.479	7.602	7.726	7.849	7.972	8.096	8.219	8.342	8.466	8.589
7	8.712	8.835	8.958	9.081	9.205	9.328	9.451	9.574	9.697	9.820
8	9.943	10.066	10.189	10.311	10.434	10.557	10.679	10.802	10.925	11.047
9	11.169	11.292	11.414	11.536	11.659	11.781	11.904	12.026	12.149	12.271
10	12.393	12.515	12.637	12.760	12.882	13.004	13.126	13.248	13.370	13.492
11	13.613	13.735	13.857	13.979	14.101	14.223	14.345	14.466	14.588	14.710
12	14.832	14.954	15.075	15.197	15.319	15.440	15.562	15.683	15.805	15.926
13	16.047	16.168	16.290	16.411	16.532	16.654	16.775	16.896	17.017	17.138
14	17.259	17.380	17.501	17.622	17.743	17.864	17.985	18.106	18.227	18.348
15	18.469	18.590	18.711	18.832	18.952	19.073	19.194	19.315	19.435	19.556
16	19.676	19.797	19.917	20.038	20.158	20.279	20.399	20.519	20.640	20.760
17	20.880	21.000	21.120	21.241	21.361	21.481	21.601	21.721	21.841	21.961
18	22.081	22.201	22.321	22.441	22.561	22.680	22.800	22.919	23.039	23.159
19	23.278	23.398	23.517	23.636	23.756	23.876	23.995	24.114	24.234	24.353
20	24.472	24.591	24.710	24.829	24.949	25.068	25.187	25.305	25.424	25.543
21	25.662	25.781	25.900	26.018	26.137	26.256	26.375	26.493	26.612	26.730
22	26.849	26.968	27.086	27.204	27.323	27.441	27.559	27.677	27.796	27.914
23	28.032	28.150	28.268	28.386	28.504	28.622	28.740	28.858	28.976	29.093
24	29.210	29.328	29.446	29.563	29.681	29.799	29.917	30.035	30.152	30.270
25	30.388	30.505	30.622	30.739	30.855	30.972	31.089	31.205	31.322	31.438
26	31.555	31.672	31.788	31.905	32.021	32.138	32.254	32.370	32.487	32.603
27	32.719	32.835	32.951	33.068	33.184	33.300	33.416	33.532	33.647	33.763
28	33.879	33.995	34.111	34.227	34.342	34.458	34.573	34.688	34.803	34.918
29	35.033	35.148	35.263	35.378	35.493	35.608	35.723	35.838	35.952	36.066
30	36.181	36.296	36.410	36.524	36.639	36.753	36.867	36.981	37.095	37.209
31	37.323	37.437	37.551	37.664	37.778	37.892	38.005	38.119	38.232	38.346
32	38.459	38.572	38.686	38.799	38.912	39.025	39.138	39.251	39.364	39.477
33	39.590	39.703	39.816	39.928	40.041	40.154	40.266	40.379	40.492	40.604
34	40.716	40.828	40.940	41.052	41.163	41.275	41.386	41.498	41.609	41.721
35	41.832	41.943	42.055	42.166	42.277	42.389	42.500	42.611	42.722	42.833
36	42.944	43.055	43.165	43.276	43.387	43.498	43.608	43.719	43.829	43.939
37	44.050	44.160	44.270	44.381	44.490	44.600	44.710	44.820	44.930	45.039
38	45.149	45.259	45.368	45.478	45.587	45.696	45.806	45.915	46.024	46.133
39	46.242	46.351	46.460	46.569	46.678	46.786	46.895	47.003	47.112	47.220
40	47.328	47.436	47.544	47.652	47.760	47.868	47.976	48.084	48.192	48.299
41	48.407	48.515	48.622	48.730	48.837	48.945	49.052	49.159	49.266	49.373
42	49.480	49.587	49.694	49.801	49.907	50.014	50.120	50.226	50.333	50.439
43	50.545	50.651	50.757	50.864	50.970	51.076	51.182	51.288	51.394	51.499
44	51.605	51.711	51.816	51.922	52.027	52.132	52.238	52.343	52.448	52.553
45	52.658	52.763	52.868	52.973	53.078	53.182	53.287	53.392	53.496	53.601
46	53.705	53.809	53.914	54.018	54.122	54.226	54.330	54.434	54.538	54.642
47	54.746	54.850	54.954	55.057	55.161	55.264	55.368	55.471	55.574	55.677
48	55.780	55.883	55.986	56.089	56.192	56.295	56.398	56.500	56.603	56.706
49	56.808	56.910	57.013	57.116	57.218	57.320	57.422	57.522	57.626	57.728
50	57.830	57.932	58.034	58.135	58.237	58.338	58.440	58.541	58.642	58.743

TABLE LXXVII.—PERCENTAGES BY VOLUME AT 60°F., CORRESPONDING TO VARIOUS PERCENTAGES BY WEIGHT IN MIXTURES OF ETHYL ALCOHOL AND WATER.—(*Continued*)

Per cent. alcohol by weight	Tenths of per cent.									
	0	1	2	3	4	5	6	7	8	9
50	57.830	57.932	58.034	58.135	58.237	58.338	58.440	58.541	58.642	58.743
51	58.844	58.945	59.046	59.147	59.248	59.349	59.450	59.550	59.651	59.752
52	59.852	59.952	60.053	60.154	60.254	60.354	60.454	60.554	60.654	60.754
53	60.854	60.954	61.054	61.154	61.254	61.353	61.453	61.552	61.652	61.751
54	61.850	61.949	62.048	62.147	62.246	62.344	62.443	62.542	62.640	62.738
55	62.837	62.936	63.034	63.132	63.231	63.329	63.427	63.526	63.624	63.722
56	63.820	63.918	64.016	64.114	64.212	64.310	64.408	64.505	64.603	64.701
57	64.798	64.895	64.993	65.090	65.188	65.285	65.382	65.479	65.575	65.672
58	65.768	65.865	65.961	66.058	66.154	66.251	66.347	66.444	66.540	66.636
59	66.732	66.828	66.924	67.020	67.116	67.212	67.308	67.404	67.499	67.595
60	67.690	67.785	67.881	67.976	68.071	68.166	68.261	68.356	68.451	68.546
61	68.641	68.736	68.831	68.925	69.020	69.115	69.209	69.304	69.398	69.492
62	69.586	69.580	69.774	69.868	69.962	70.056	70.149	70.243	70.336	70.430
63	70.523	70.616	70.710	70.803	70.897	70.990	71.083	71.176	71.269	71.362
64	71.455	71.548	71.641	71.733	71.826	71.918	72.011	72.103	72.196	72.288
65	72.380	72.472	72.564	72.656	72.748	72.840	72.932	73.024	73.116	73.207
66	73.299	73.391	73.482	73.574	73.665	73.756	73.847	73.938	74.029	74.120
67	74.211	74.302	74.393	74.484	74.575	74.665	74.756	74.846	74.937	75.027
68	75.117	75.207	75.297	75.387	75.477	75.567	75.657	75.747	75.837	75.926
69	76.016	76.106	76.195	76.285	76.374	76.464	76.553	76.642	76.731	76.820
70	76.909	76.998	77.087	77.176	77.264	77.353	77.441	77.529	77.618	77.706
71	77.794	77.882	77.970	78.058	78.146	78.234	78.322	78.410	78.497	78.584
72	78.672	78.760	78.847	78.934	79.022	79.109	79.196	79.283	79.370	79.457
73	79.544	79.631	79.718	79.805	79.892	79.978	80.065	80.151	80.238	80.324
74	80.410	80.496	80.582	80.668	80.754	80.840	80.926	81.012	81.098	81.183
75	81.269	81.355	81.440	81.526	81.611	81.696	81.781	81.866	81.951	82.036
76	82.121	82.206	82.291	82.376	82.461	82.545	82.630	82.714	82.799	82.883
77	82.967	83.051	83.135	83.219	83.303	83.387	83.471	83.555	83.638	83.722
78	83.805	83.888	83.972	84.055	84.138	84.221	84.304	84.387	84.470	84.553
79	84.636	84.719	84.801	84.884	84.966	85.049	85.131	85.213	85.295	85.377
80	85.459	85.541	85.623	85.705	85.787	85.868	85.950	86.031	86.113	86.194
81	86.275	86.356	86.437	86.518	86.599	86.680	86.761	86.842	86.922	87.002
82	87.083	87.164	87.244	87.324	87.405	87.485	87.565	87.645	87.725	87.805
83	87.885	87.965	88.045	88.124	88.204	88.283	88.362	88.441	88.520	88.599
84	88.678	88.757	88.836	88.915	88.994	89.072	89.151	89.229	89.308	89.386
85	89.464	89.542	89.620	89.698	89.776	89.854	89.932	90.009	90.086	90.163
86	90.240	90.317	90.394	90.471	90.548	90.625	90.702	90.779	90.855	90.932
87	91.008	91.084	91.161	91.237	91.313	91.388	91.464	91.540	91.615	91.691
88	91.766	91.841	91.917	91.992	92.068	92.143	92.218	92.293	92.367	92.442
89	92.517	92.591	92.666	92.740	92.814	92.888	92.962	93.035	93.108	93.181
90	93.254	93.327	93.400	93.473	93.546	93.619	93.692	93.764	93.837	93.910
91	93.982	94.054	94.126	94.198	94.270	94.342	94.414	94.486	94.557	94.629
92	94.700	94.771	94.842	94.913	94.984	95.055	95.126	95.196	95.267	95.337
93	95.407	95.477	95.547	95.617	95.687	95.757	95.826	95.896	95.965	96.034
94	96.103	96.172	96.241	96.310	96.378	96.447	96.515	96.583	96.652	96.720
95	96.787	96.855	96.922	96.990	97.057	97.125	97.192	97.259	97.326	97.392
96	97.459	97.526	97.592	97.658	97.724	97.790	97.856	97.921	97.987	98.052
97	98.117	98.182	98.247	98.312	98.376	98.440	98.505	98.569	98.633	98.696
98	98.759	98.822	98.886	98.949	99.012	99.075	99.137	99.200	99.262	99.324
99	99.386	99.448	99.510	99.572	99.633	99.695	99.756	99.817	99.878	99.939
100	100.000									

TABLE LXXVIII.—PERCENTAGES BY WEIGHT, CORRESPONDING TO VARIOUS PERCENTAGES BY VOLUME AT 60°F. IN MIXTURES OF ETHYL ALCOHOL AND WATER

Per cent. alcohol by volume at 60°F.	Per cent. alcohol by weight	Differences	Per cent. alcohol by volume at 60°F.	Per cent. alcohol by weight	Differences
0	0.000		50	42.487	
1	0.795	0.795	51	43.428	0.941
2	1.593	.798	52	44.374	.946
3	2.392	.799	53	45.326	.952
4	3.194	.802	54	46.283	.957
		.804			.962
5	3.998		55	47.245	
6	4.804	.806	56	48.214	.969
7	5.612	.808	57	49.187	.973
8	6.422	.810	58	50.167	.980
9	7.234	.812	59	51.154	.987
		.813			.993
10	8.047		60	52.147	
11	8.862	.815	61	53.146	.999
12	9.679	.817	62	54.152	1.006
13	10.497	.818	63	55.165	1.013
14	11.317	.820	64	56.184	1.019
		.821			1.024
15	12.138		65	57.208	
16	12.961	.823	66	58.241	1.033
17	13.786	.825	67	59.279	1.038
18	14.612	.826	68	60.325	1.046
19	15.440	.828	69	61.379	1.054
		.829			1.062
20	16.269		70	62.441	
21	17.100	.831	71	63.511	1.070
22	17.933	.833	72	64.588	1.077
23	18.768	.835	73	65.674	1.086
24	19.604	.836	74	66.768	1.094
		.839			1.102
25	20.443		75	67.870	
26	21.285	.842	76	68.982	1.112
27	22.127	.842	77	70.102	1.120
28	22.973	.846	78	71.234	1.132
29	23.820	.847	79	72.375	1.141
		.850			1.151
30	24.670		80	73.526	
31	25.524	.854	81	74.686	1.160
32	26.382	.858	82	75.858	1.172
33	27.242	.860	83	77.039	1.181
34	28.104	.862	84	78.233	1.194
		.867			1.208
35	28.971		85	79.441	
36	29.842	.871	86	80.662	1.221
37	30.717	.875	87	81.897	1.235
38	31.596	.879	88	83.144	1.247
39	32.478	.882	89	84.408	1.264
		.886			1.281
40	33.364		90	85.689	
41	34.254	.890	91	86.989	1.300
42	35.150	.896	92	88.310	1.321
43	36.050	.900	93	89.652	1.342
44	36.955	.905	94	91.025	1.373
		.910			1.398
45	37.865		95	92.423	
46	38.778	.913	96	93.851	1.428
47	39.697	.919	97	95.315	1.464
48	40.622	.925	98	96.820	1.505
49	41.551	.929	99	98.381	1.561
		.936			1.619
50	42.487		100	100.000	

LXXIV or Table LXXV may be found the corresponding value for per cent. by weight, which may then be converted into per cent. by volume by means of Table LXXVII. Remember that the value taken from the table is in any case only the percentage of alcohol in the distillate and must still be calculated to the original sample.

Notes.—The quantitative separation of pure alcohol from any food material is practically an impossibility. Hence, the methods commonly used depend upon the examination of a mixture of alcohol and water, separated by distillation from the original material. Methods of examining the distillate other than the determination of its specific gravity comprise the determination of its refractive index, its boiling point, or chemical tests, such as oxidation to acetic acid by some suitable oxidizing agent. Of these, the first is described under the determination of methyl alcohol, the second is in general suited only for approximate work, and the last is used only for the estimation of small amounts. For these reasons the specific gravity method is the only one considered here.

The addition of calcium carbonate or of sodium hydroxide is for the purpose of neutralizing any volatile acid, as acetic, which might be present as the result of fermentation and would otherwise pass into the distillate with the alcohol and water. In many cases the neutralizing will not be necessary.

If the sample contains volatile oils, these will, of course, pass into the distillate and either the method described on page 401 or that given on page 407 should be used instead of simple distillation.

The result is expressed ordinarily in per cent. of absolute alcohol by weight or by volume. The former is perhaps more in keeping with the general method of expressing analytical results, but the statement as per cent. by volume is required under many of the liquor laws, and under the Federal Pure Food Law, hence is probably more commonly used. Still another method of stating the alcohol content, and that employed in the Bureau of Internal Revenue, is as the percentage of *proof spirit*, by which is meant a liquor containing 50 per cent. by volume of alcohol. Thus, a whiskey of "96 proof" would contain 48 per cent. of alcohol by volume. *British proof spirit* is somewhat

different, containing 57.07 per cent. of alcohol by volume at 15.6°C. The percentage of alcohol by weight in the distillate can, of course, be taken directly from the appropriate table. In order to calculate it to the original sample the weight or the specific gravity of the latter must be also known.

As a result of the use of different alcohol tables, based on varying data, some confusion has resulted, since the results obtained may be slightly different. Those given here, published by the Bureau of Standards,[1] have been adopted provisionally by the Association of Official Agricultural Chemists in the attempt to unify methods. Since in the construction of the tables all weighings have been reduced to *vacuo*, the weights of distillate and of water contained in the pyknometer should for exact work be corrected for the buoyant effect of the air. This may be done in each case by adding 0.00106 gram for each gram of water or of distillate that the pyknometer apparently contains. Since, however, this correction is applied to both the distillate and the water its effect on the specific gravity is very slight and can be neglected for all ordinary work.

Detection of Methyl Alcohol.—The occasional use has been reported in food products of methyl alcohol in place of the more costly ethyl alcohol. This is most likely to occur in such products as flavoring extracts or distilled liquors and might be due to the use of "denatured" alcohol rather than to the direct addition of methyl alcohol itself.

Various methods for the detection of methyl alcohol in the presence of ethyl alcohol have been proposed, but it will suffice to describe only two as especially applicable in food analysis, the first because it is rapid and requires no special apparatus, the second because it is both qualitative and quantitative. Either method should be applied to the distillate obtained in the determination of alcohol as described above.

(*a*) **U. S. Pharmacopœia Test.**[2] Place 10 cc. of the liquid to be tested, having an alcohol concentration of about 10 per cent., in a test-tube surrounded by cold water. Wind a length of

[1] Bureau of Standards, *Circular* **19** (3d Ed.).

[2] Mulliken and Scudder: *Am. Chem. J.*, **1899**, 266; **1900**, 444; U. S. Pharmacopœia, 8th Decennial Revision, p. 34.

medium sized copper wire around a pencil so that the closely coiled spiral shall form a cylinder about an inch long, leaving about 8 in. unwound for a handle. Heat the spiral red hot in the oxidizing flame of a burner, plunge it to the bottom of the test-tube and hold it there for 1 to 2 seconds. Repeat this five or six times. Filter the solution and boil it gently until any odor of acetaldehyde is no longer apparent. Cool the solution, add one drop of a 1:200 aqueous solution of resorcin and pour it cautiously on top of 5–10 cc. of concentrated sulphuric acid in a test tube. Allow to stand for 3 minutes, then shake *very gently* for a minute, causing a very gradual mixing of a portion of the two layers. With 2 per cent. of methyl alcohol a rose-red ring will appear at the junction of the liquids, and if the quantity is somewhat greater than this characteristic rose-red flocks will separate when the tube is gently shaken.

Notes.—The treatment with the hot spiral oxidizes a part of the alcohol to aldehyde, the methyl alcohol to formaldehyde and the ethyl alcohol to acetaldehyde. By the boiling the acetaldehyde (and some of the formaldehyde) is expelled, thus increasing the delicacy of the test, since acetaldehyde gives with resorcin a yellowish-brown ring and flocks, which tend to obscure the formaldehyde reaction.

Other methods for the detection of formaldehyde, such as are described on pages 89 and 124, might be employed, but care should be taken not to use too delicate a test since traces of formaldehyde are produced by the oxidation of ethyl alcohol. For this reason the gallic acid test (page 90), an extremely characteristic test for formaldehyde and one not readily obscured by the presence of other compounds, is inadmissible.

The test as described, even including the production of the more characteristic flocks, is probably delicate enough to detect methyl alcohol in the quantities in which it would be likely to be added to a food product for commercial profit, but if a negative result is obtained and there is still reason to believe that methyl alcohol may be present, a portion of the alcoholic liquid may be fractionated several times through a Glinsky, Hempel or other suitable apparatus and the test repeated on the concentrated solution. The test may also be made more delicate by conducting

the oxidation with a solution of chromic acid[1] instead of solid copper oxide in wire form.

The methods for the detection of methyl alcohol adopted as provisional by the Association of Official Agricultural Chemists[2] are those of Trillat[3] and of Riche and Bardy.[4] These are, however, long and tedious and suited only to experienced chemists, hence are omitted here in favor of the shorter and simpler method described. A good critical summary of the more important tests for detecting methyl alcohol has been published by Scudder.[5]

(*b*) **Refractometer Method.**—The densities of pure methyl alcohol and of ethyl alcohol are not far apart, the former being 0.7965 and the latter 0.7939 at $\frac{15^\circ}{15^\circ}$C. The refractive indices, however, are quite different, being 1.3614 at 20°C. for ethyl alcohol and 1.3281 at the same temperature for methyl alcohol, a difference easily measured. It has been proposed[6] to utilize this distinction for the detection of methyl alcohol in ethyl alcohol, the refraction being determined by means of the immersion refractometer.

To do this use the distillate obtained in determining the per cent. of alcohol from the specific gravity (page 416) and determine its reading on the immersion refractometer at 20°C. The following table, worked out by Leach and Lythgoe, shows the scale reading on the refractometer for each per cent. of methyl and ethyl alcohol present as determined from the density. If the refractometer reading is appreciably lower than corresponds to the per cent. of ethyl alcohol shown by the density, the presence of methyl alcohol is indicated.

Moreover, the addition of methyl to ethyl alcohol lowers the refraction in direct proportion to the amount added. Hence, the quantitative determination may be made by interpolation in Table LXXIX using the figures for pure ethyl and methyl alcohol of the same alcoholic strength as the distillate.

[1] Vorisek: *J. Soc. Chem. Ind.*, **1909,** 823; Bacon: *Bur. of Chem., Circular* **74.**

[2] *Bur. of Chem., Bull.* **107** (Rev.), p. 99.

[3] *Compt. rend.*, **1898,** 232; *Analyst*, **1899,** 211.

[4] *Compt. rend.*, **1875,** 1076; Leach: Food Analysis, 3d Ed., p. 751.

[5] *J. Am. Chem. Soc.*, **1905,** 892.

[6] Leach and Lythgoe: *J. Am. Chem. Soc.*, **1905,** 964.

Example.—Suppose the distillate in an alcohol determination has a density at $\frac{20°}{4°}$C. of 0.97076, corresponding to 18.40 per cent. of ethyl alcohol by weight, and has a refraction of 35.8 on the immersion refractometer at 20°C. By interpolation in Table LXXIX the readings of ethyl and methyl alcohol corresponding to 18.40 per cent. are 47.3 and 25.4, respectively, the difference being 21.9.

$$47.3 - 35.8 = 11.5; \frac{11.5}{21.9} \times 100 = 52.5$$

Hence 52.5 per cent. of the alcohol present is methyl.

Note.—The difference in refraction for the two alcohols, as will be seen from the table, varies considerably for different strengths. In the case of methyl alcohol the refraction increases with increasing concentration until it reaches a maximum at about 50 per cent. by weight. In the case of ethyl alcohol the maximum refraction is reached at 75 per cent., but the decrease above this concentration is by no means as rapid as with methyl alcohol. This means that the delicacy of the method is considerably greater for the higher concentrations of alcohol, at 90 per cent. strength the method being capable of showing 0.1 per cent. of methyl alcohol with accuracy. In the case of most food products, however, the necessity for distilling without loss of alcohol restricts the method to the lower concentrations of alcohol.

Wine

The term *wine*, without further qualification, is universally understood to mean the product of alcoholic fermentation of the juice of the grape. This not only excludes the fermented product of other fruit juices, but when the definition is made more rigid, as in a legal standard, it is usually specified that the wine shall be made by the "usual cellar treatment" (see page 453). The purpose of this is not only to restrict the original crude material to the grape juice, but also to limit all additions to those substances which occur naturally in sound grapes, or which experience has shown to be of distinct benefit to the flavor or keeping qualities of the wine.

TABLE LXXIX.—SCALE READINGS ON ZEISS IMMERSION REFRACTOMETER AT 20°C., CORRESPONDING TO EACH PER CENT. BY WEIGHT OF METHYL AND ETHYL ALCOHOLS

Per cent. alcohol by weight	Scale readings		Per cent. alcohol by weight	Scale readings		Per cent. alcohol by weight	Scale readings		Per cent. alcohol by weight	Scale readings	
	Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol
0	14.5	14.5	26	30.3	61.9	51	39.7	91.1	76	29.0	101.0
1	14.8	16.0	27	30.9	63.7	52	39.6	91.8	77	28.3	100.9
2	15.4	17.6	28	31.6	65.5	53	39.6	92.4	78	27.6	100.9
3	16.0	19.1	29	32.2	67.2	54	39.5	93.0	79	26.8	100.8
4	16.6	20.7	30	32.8	69.0	55	39.4	93.6	80	26.0	100.7
5	17.2	22.3	31	33.5	70.4	56	39.2	94.1	81	25.1	100.6
6	17.8	24.1	32	34.1	71.7	57	39.0	94.7	82	24.3	100.5
7	18.4	25.9	33	34.7	73.1	58	38.6	95.2	83	23.6	100.4
8	19.0	27.8	34	35.2	74.4	59	38.3	95.7	84	22.8	100.3
9	19.6	29.6	35	35.8	75.8	60	37.9	96.2	85	21.8	100.1
10	20.2	31.4	36	36.3	76.9	61	37.5	96.7	86	20.8	99.8
11	20.8	33.2	37	36.8	78.0	62	37.0	97.1	87	19.7	99.5
12	21.4	35.0	38	37.3	79.1	63	36.5	97.5	88	18.6	99.2
13	22.0	36.9	39	37.7	80.2	64	36.0	98.0	89	17.3	98.9
14	22.6	38.7	40	38.1	81.3	65	35.5	98.3	90	16.1	98.6
15	23.2	40.5	41	38.4	82.3	66	35.0	98.7	91	14.9	98.3
16	23.9	42.5	42	38.8	83.3	67	34.5	99.1	92	13.7	97.8
17	24.5	44.5	43	39.2	84.2	68	34.0	99.4	93	12.4	97.2
18	25.2	46.5	44	39.3	85.2	69	33.5	99.7	94	11.0	96.4
19	25.8	48.5	45	39.4	86.2	70	33.0	100.0	95	9.6	95.7
20	26.5	50.5	46	39.5	87.0	71	32.3	100.2	96	8.2	94.9
21	27.1	52.4	47	39.6	87.8	72	31.7	100.4	97	6.7	94.0
22	27.8	54.3	48	39.7	88.7	73	31.1	100.6	98	3.5	93.0
23	28.4	56.3	49	39.8	89.5	74	30.4	100.8	99	3.5	92.0
24	29.1	58.2	50	39.8	90.3	75	29.7	101.0	100	2.0	91.0
25	29.7	60.1									

Classification.—On account of the exceedingly great variation in the character of the grapes grown in different soils and in localities widely separated, to say nothing of the changes in appearance, flavor and composition brought about by differences in the methods of manufacture, wines are placed on the market in almost infinite variety. Many of these are distinguished and well known in commerce by the name of a region, a locality or a particular vineyard; the wines of a certain vineyard may be further differentiated according to the year of the vintage; finally, the special brands and names which appear may be numbered among

the thousands. With such trade distinctions, however, it is not necessary to deal for the purposes of analysis. Based on essential differences in chemical composition, natural wines may be grouped into three divisions, each including two sub-divisions, red and white, depending on whether the color is extracted from the skins of the grapes or not:

(*a*) Dry wines.
(*b*) Sweet wines.
(*c*) Sparkling wines.

A *dry wine* is one in which practically all of the sugar, certainly all that can be perceived by the taste, has been changed by fermentation. A *sweet wine* is one in which sufficient sugar remains in the finished product to give it a noticeable sweet taste. This difference in the sugar content of the wine is intentional and is due to the desire of the manufacturer in one case to carry on the fermentation continuously until all the sugar has been converted to the maximum amount of alcohol and carbon dioxide, while in the latter case the progress of the fermentation is stopped by the addition of alcohol while part of the sugar still remains unchanged. Dry wines very seldom are absolutely free from sugar, the amount ranging from a few hundredths up to several tenths of 1 per cent. Sweet wine contains, of course, much more, ranging from 2 or 3 up to 10 per cent. or more. The Federal standards (see page 453) have set as an arbitrary line between dry and sweet wines the presence of 1 gram of sugars per 100 cc.

A *sparkling wine* is one which is charged with an excess of carbon dioxide, usually enough to cause a pressure of several atmospheres in the bottle at ordinary temperature. With natural wines this condition is brought about by a supplementary fermentation in the bottle; with artificial wines it may be due to carbonating, in a similar manner to the production of effervescent drinks. The opposite term to sparkling wine is *still wine*, but this is naturally included in the two classes just considered.

Another classification, which is at times of value in considering the analytical characteristics of wine, is into *natural* and *fortified* wines. The former are those to which neither sugar nor alcohol has been added but in which the fermentation has been allowed to proceed until checked naturally by lack of sugar or increase of

alcoholic strength. The dry wines are typical of this class. The fortified wines have had alcohol, either in the form of brandy (wine distillate) or from some other source, added. This addition ordinarily takes place before the fermentation is finished, so that a certain proportion of the sugar is left unfermented and the wine is a sweet wine. Wines of this type may contain 20 per cent. or more of alcohol, while the natural fermentation does not yield over 14.5.

Manufacture.—Only the merest outline of the methods used in producing wine, enough to give a general survey of the chemical changes brought about, can be given here.

(*a*) *Pressing.*—The grapes are picked, usually at the time when they contain the most sugar, and carted to the winery for pressing. If the grapes are to be used for the production of white wine they are crushed and pressed so that only the juice shall be fermented; if for red wine the entire pulp after crushing is placed in the fermenting vats in order to extract the color during fermentation. The crushing and pressing are done by rollers and screw or hydraulic presses, the juice which is pressed out constituting the *must* and the residue left in the press the *pomace* or *marc*.

(*b*) *Fermenting.*—The alcoholic fermentation of the must is due to the presence of various yeasts, of which the most important is the so-called "true wine yeast," *Saccharomyces ellipsoideus*. The *zymase*, or enzyme present in the yeast cells, acts on the dissolved sugar, changing it to alcohol and carbon dioxide. These yeasts are present on the skins of the grapes, so that under ordinarily favorable conditions the must will begin to ferment almost immediately, as shown by the increase in temperature, formation of gas bubbles and change from a sweet taste to an acid and alcoholic one. On account, however, of the presence of other less desirable yeasts the wine maker often adds to the must pure cultures of special yeasts, thereby securing better control of the fermentation and a cleaner product.

The fermentation is carried on in casks or vats at a temperature varying between 55° and 85°F., the temperature and details of the process differing somewhat for white and for red wines. After fermenting the wine is drawn off into other casks, frequently sulphured to prevent the further development of micro-

organisms, and allowed to ripen for several months or until the following autumn, being occasionally drawn or "racked" off into other containers in order to remove the sediment and by oxidation hasten the precipitation of undesirable albuminous substances and improve the flavor. "Finings," usually gelatin or egg albumen, may be added in small quantity to assist in this precipitation and clarification.

(*c*) *Bottling.*—When the wine has ceased forming a deposit, is bright and clear and has reached its optimum quality it is bottled, the object being to prevent further deleterious change by protecting it from oxidation or the action of microorganisms. After bottling the wine improves for several months, and then remains with little change except a very gradual development of the highest qualities of flavor for many years, if kept under suitable conditions.

Sparkling Wine.—The preliminary fermentation of sparkling wines is carried out in practically the manner described above. After blending, sufficient sugar solution is added to develop a pressure of about five atmospheres during the subsequent bottle fermentation, special yeast is added and the wine bottled and corked. At the end of the bottle fermentation, which lasts from 6 months to 10 years, the bottles are gradually inverted in the racks, thus collecting the sediment near the cork. By dexterously uncorking the bottle momentarily this sediment is removed. Some more sugar is often added and the bottle again securely corked.

General Composition.—The principal ingredients present in the must are sugar (dextrose); organic acids, principally tartaric, malic and tannic; albuminoids; dextrin, pectin and other mucilaginous carbohydrates; and small amounts of various flavoring substances. Of these, the sugar may almost entirely disappear during fermentation or a considerable proportion may remain in the finished wine. The tartaric acid, which varies considerably in amount between red and white wines, is largely removed by the precipitants used in the fining process. In addition, part of the acids may serve as food for microorganisms during the process of manufacture and a small proportion of acetic acid may be formed by subsidiary fermentations. The albuminoids are largely removed, partly as nourishment for the yeast and partly

by precipitation during the fermentation and cellar treatment. The mucilaginous substances are largely precipitated as the fermentation proceeds on account of their relative insolubility in dilute alcohol.

The finished wine, then, while retaining in part the character of the must, will have the proportions of the constituents largely altered and contain some new ones. From a chemical standpoint its important constituents are: Alcohol, glycerin, sugar, acids, both fixed and volatile, tannin and color.

Forms of Adulteration.—The most common methods of adulterating wine consist either in some improper treatment during the process of manufacture, the addition of some improper substance to the wine itself, or the substitution of an inferior or less desirable product for the genuine article.

With reference especially to materials added during the process of manufacture, the central idea of all proper additions is that they should be such as to improve the product and not to defraud the purchaser. One thing is *amelioration* or improvement of the wine; the other is *adulteration*.

The additions which are commonly allowed in wine are for the purposes of controlling the fermentation or character of the wine, or for correcting natural defects due to climatic or seasonal conditions. Of these the most important are:[1]

The usual agents, such as tannin, albumen, casein or gelatin, which are used as "fining" agents in the clarification of the wine;

Sulphurous acid or bisulphites in limited quantities (see page 462) for the control of the fermentation and to assist in keeping the wine;

In the case of excessive acidity, neutralizing agents which do not render the wine injurious to health, such as neutral potassium tartrate or calcium carbonate;

In the case of deficient acidity, tartaric acid;

In the case of deficiency in saccharine matter, condensed grape must, or a pure dry sugar.

The addition of grape brandy for the fortification of sweet wine is also permitted in accordance with the Sweet Wine Fortification Act of June 7, 1906.

[1] Food Inspection Decision 156.

Additions which would be regarded as constituting adulteration would be such as adding water to increase the bulk of the product, the addition of ordinary alcohol or adding fermented liquids derived from other sources than the grape. The addition of brandy, even, must be only to certain classes of wines and then under definite restrictions. (See page 455.) Such additions as artificial preservatives or color would naturally constitute adulteration under the general provisions of the Food and Drugs Act, except in a few instances, when they may be added if properly declared.

The addition of gypsum or plaster of Paris to the wine is also generally regarded as adulteration, and either it is forbidden or the amount which may be used is strictly limited. The object of this practice, commercially known as "plastering," is to improve the wine by precipitating a certain proportion of the potassium acid tartrate as the insoluble calcium salt, thus bringing about a sort of clarification which improves the color and keeping qualities of the wine. The increased amount of potassium sulphate thus added is, however, considered decidedly objectionable.[1]

The practice is also in vogue of adding to the "marc" or "pomace," that is, the residue of pulp, skins, seeds, etc., from which the must has been drawn off, a solution of sugar in water. This readily ferments and yields a considerable quantity of "wine" which is used for blending or sometimes, by the help of saccharin, coloring matter and preservatives, is put on the market directly. It is required that such a product shall be so labeled as to indicate its true character.

Some forms of adulteration are possibly better described as substitution or misbranding. The use of raisins in the manufacture yields a wine which from the nature of the raw material would be quite difficult to distinguish from a product made by the fermentation of fresh grapes. It is required that such a wine be so labeled as to indicate its origin, but the problem of detecting it is not an easy one so far as chemical tests are concerned, because the constituents of raisins are for the most part

[1] According to Neufeld (*Z. Nahr. Genussm*, **1914** (*27*), 299) the presence of sulphates, calculated as potassium sulphate, in excess of 2 grams per liter has been shown to be distinctly harmful physiologically.

the same as those of grapes, and the solution obtained by soaking them in water can be fermented to yield a very similar product to ordinary wine. Such a product, however, can usually be detected by its characteristic flavor, this being readily distinguished by those familiar with the taste of genuine wines.

Misbranding as to variety, that is, so labeling an inferior product as to indicate that it is made from a standard or more highly prized variety of grape, is hardly to be detected by chemical tests, but must usually be shown by differences in character and flavor which are apparent to the trained palate. The labeling of a domestic wine as being a foreign product can often be detected by analysis, since the wines produced in this country are in many cases distinctly different in their exact composition from the foreign product which they are made to resemble. Such wines must be labelled with the word "TYPE" or with the name of the State or locality where produced.

METHODS OF ANALYSIS

Statement of Results.—The most convenient form in which to express the analytical results is as grams per 100 cc. of wine, and unless some statement is made to the contrary they should be so stated in the determinations that follow. If the *per cent.* of any constituent is desired this can be readily calculated from the specific gravity of the sample.

Specific Gravity.—This may be determined at 15.6°C. by means of the pyknometer or Westphal balance as described under General Methods, pages 1 to 4.

Alcohol.—Use 100 cc. of the sample and determine the alcohol as described on page 416, noting the directions in regard to neutralizing the acidity if necessary. If the sample froths badly this may be prevented by the addition of a little tannic acid, which precipitates the proteins. Calculate the result as per cent. of alcohol by volume. If it is desired to know also the grams of alcohol per 100 cc., as for calculating alcohol ratios in interpreting the results, this may be found by multiplying the per cent. by volume by the specific gravity of absolute alcohol.

Extract.—On account of the presence of levulose in considerable proportions in sweet wines the method to be followed for

determining the total solids or extract varies somewhat with the character of the sample.

Method.—(*a*) *By Calculation.*—Calculate the extract from the specific gravity of the alcohol-free wine by the formula:

$$E = \frac{s - s'}{0.00386}$$

in which s is the specific gravity of the wine, s' the specific gravity of the distillate obtained in the determination of alcohol and 0.00386 the increase in gravity caused by 1 gram of wine solids in 100 cc. E will be the extract in grams per 100 cc.

(*b*) *By Direct Evaporation.*—If the extract, calculated as above, is less than 3 grams per 100 cc. it should be determined directly. Evaporate 50 cc. of the wine on the water-bath to a sirupy consistency in a flat-bottomed platinum dish. Dry in the oven at 100°C. for 2½ hours, cool and weigh.

If the extract, as calculated, is between 3 and 4 grams per 100 cc., evaporate such a quantity of the wine that the weight of residue shall not exceed 1.5 grams.

If the calculated extract is over 4 grams per 100 cc., the result obtained by method (*a*) should be accepted and no direct determination made.

Notes.—With sweet wines the drying method is inaccurate on account of the decomposition of levulose at temperatures much above 75°C. (See also page 273.)

The calculation by the factor 0.00386 is based on the assumption that this factor, which is the increase in density caused by 1 gram of sucrose in 100 cc. of water, is correct for the solids of wine. This assumption is reasonably correct for sweet wine, in which the extract consists largely of sugars, but is less exact for dry wines, in which the extract consists mainly of non-sugars. In spite of this, the Swiss Association of Analytical Chemists has recommended[1] that the indirect method be employed in all wines on account of the varying results obtained in the direct drying, these varying much with the dish and oven employed.

The specific gravity of the alcohol-free wine may also be determined by evaporating a measured quantity of the wine on the

[1] *Mitt. d. Schweizer. Ges.-Amte.*, **1911**, 447.

water-bath to one-fourth its volume, diluting with water to the original volume and determining the gravity as usual.

Ash.—Use the residue obtained in the determination of extract, or if this was not determined by direct drying evaporate to dryness 25 cc. of the wine. In either case cautiously char the residue but do not ignite strongly, because of the danger of loss of alkali chlorides and of fusing potassium carbonate. Boil the charred mass several times with small portions of water, decanting each time through an ashless filter, place the filter in the dish and ignite until the ash is white. Add the filtrate to the dish, evaporate to dryness on the water-bath, moisten with ammonium carbonate solution, ignite at a low red heat, cool and weigh. (See also page 17.)

Note.—On account of the presence of potassium salts in considerable amounts, the charring of the ash must be done with great care. The addition of ammonium carbonate is to restore the carbon dioxide to carbonates that have been decomposed by the ignition.

Acidity.—Total Free Acids.—*Method (a):* Heat 25 cc. of the wine quickly to incipient boiling and titrate at once with $\frac{N}{2}$ or $\frac{N}{4}$ sodium hydroxide, using delicate litmus paper as indicator. The end-point is reached when a drop of the liquid will no longer produce a red spot on the dry blue litmus paper. It will be found a saving of time to make a preliminary titration to ascertain the acidity approximately before proceeding with the final titrations. The alkali should be standardized against tartaric acid in the same manner. Calculate the total acidity as tartaric acid, $H_2C_4H_4O_6$.

Method (b):[1] Measure 10 cc. of wine into a 500 cc. flask, add 200 cc. of distilled water and boil 3 minutes under a reflux condenser. After cooling rinse out the condenser tube with a little distilled water, add 3–5 drops of phenolphthalein and titrate with $\frac{N}{10}$ sodium hydroxide. Titrate carefully, adding the standard alkali slowly toward the last, until 2 or 3 drops produce no perceptible change. Note the color changes by holding the flask a short

[1] Bretan: Guide pratique des falsifications des substances alimentaires, p. 318; Hortvet: *Bur. of Chem., Bull.* **122,** p. 13; **132,** p. 71.

distance above a well-lighted white surface. If the sample is very dark colored it may be necessary to dilute still further before boiling.

In the same way boil and titrate 210 cc. of distilled water and calculate the total acids from the difference between the two titrations, reporting the result as tartaric acid as above.

Notes.—The wine is heated before titrating, partly to expel carbon dioxide and partly to decrease the amphoteric effect of the phosphates and albuminates on the indicator. If phenolphthalein is used it is of course necessary to heat the sample actually to boiling, which is best done with the necessary precaution to avoid loss of volatile acids (principally acetic). The use of $\frac{N}{2}$ or $\frac{N}{4}$ alkali is to shorten the time necessary for titration and thus lessen the danger of loss of acid.

Method (*a*) is the older and the one in common use, but the second method is with many wines easier to carry out and gives more uniform results, usually somewhat higher, the results in any case being largely dependent on the particular indicator employed.

The results are calculated as tartaric acid largely through force of custom, this being the principal acid present, though not necessarily as the free acid itself. Other ways of stating the result are as grams of sulphuric acid per liter or as cc. of normal acid in 100 cc. of wine.

Volatile Acids.—(*a*)[1] Measure 50 cc. of wine into a 250 cc. round-bottomed flask and distil by means of a current of steam introduced by a tube reaching to the bottom of the flask, heat also being applied by a small flame in order to reduce the volume of the liquid and keep it constant at about 25 cc. If much foaming is experienced a little tannin may be added. Distil 200 cc. and titrate with $\frac{N}{10}$ sodium hydroxide and phenolphthalein. Calculate the acidity as acetic acid, $HC_2H_3O_2$.

Note.—This is the longest established method for determining volatile acids in wine and is perhaps still the most widely used. The results, however, show very considerable differences at times, due to variations in the apparatus employed and to the

[1] Lindemann: *Z. anal. Chem.*, **1883**, 166; *Bur. of Chem., Bull.* **107**, p. 86.

direct heating of the sample during the steam distillation. Further, it has been shown by numerous observers[1] that not all of the volatile acids are distilled in 200 cc. of distillate except under rigidly prescribed conditions. On the other hand, if larger volumes of distillate are collected the process becomes tedious and there is danger of distilling notable quantities of lactic acid.[2]

Many of these difficulties are avoided and the time shortened by having the distilling flask surrounded by boiling water as in the method described below.

(*b*) *Hortvet Method.*[3]—The apparatus (Fig. 59) which may be secured from dealers in chemical supplies, consists of a 300 cc.

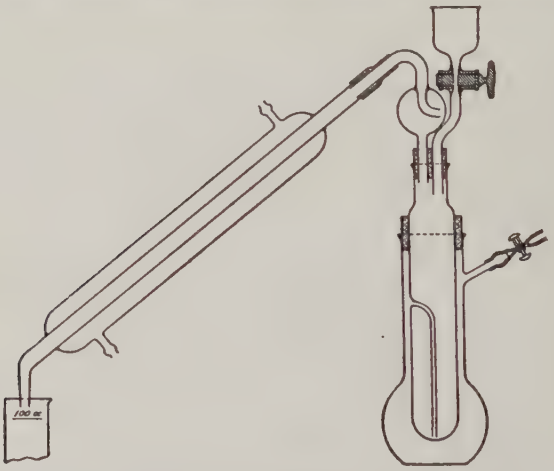


FIG. 59.—Hortvet's apparatus for volatile acids.

flask into the neck of which is fitted a stoppered cylindrical flask with a steam tube sealed in at the side, and carrying a funnel tube and a delivery tube provided with a safety bulb.[4]

To make a determination, pour 150 cc. of recently boiled

[1] Among others see Windisch and Roettgen: *Z. Nahr. Genussm.*, **1905,** 70; **1911,** 155; Hortvet: *J. Ind. Eng. Chem.*, **1909,** 31.

[2] Kulisch: *Z. Nahr. Genussm.*, **1907,** 663.

[3] Hortvet: *Loc. cit.*

[4] A somewhat more elaborate form of this apparatus, using a copper outside flask and a constant flow of water, has been described by Gore (*Bur. of Chem.*, Circular **44**).

water into the larger flask, attach the cylindrical flask by means of a short section of stout rubber tubing, add 10 cc. of wine (previously freed from carbon dioxide by shaking) and heat to boiling, having the pinch cock at the side of the larger flask open. When the water has boiled for a moment, close the pinch cock and distil until 100 cc. have been collected. Titrate as above.

Note.—The fixed acids may be determined, if desired, on the same sample by cooling the flask after the distillation is completed, thus draining the wine residue into the larger flask, rinsing with boiled water and titrating as under Total Acids (*b*), page 438.

(*c*) *Indirect Method.*—If the apparatus for methods (*a*) and (*b*) is not available approximate results may be obtained by the indirect method.[1] Evaporate 25 cc. of the wine in a porcelain dish on the water-bath to about 3–5 cc., dissolve the residue in 25 cc. of hot water, again evaporate to 3–5 cc. and repeat once more, making three evaporations in all. Finally, dissolve the residue in hot water and titrate the acids as described under Total Acids (*a*), page 438. From the difference between this titration and the total acids calculate the volatile acids.

Note.—The indirect method in general is inexact on account of the decomposition of acid salts and change of acids during the heating. The modification described here, in which the sample is never heated above the temperature of the water-bath and not evaporated to dryness, minimizes these errors and gives fairly concordant and satisfactory results.

Fixed Acids.—These are best determined by difference from the determinations of total acids and volatile acids, although they are determined directly in method (*c*) for volatile acids above. Calculate in terms of tartaric acid, $H_2C_4H_4O_6$, and if calculating by difference remember that the total acids have been calculated as tartaric and the volatile acids as acetic.

Tartaric Acid and Tartrates.—(*a*) **Total Tartaric Acid.**[2]—To 100 cc. of wine add 2 cc. of glacial acetic acid, 3 drops of a 20 per cent. solution of potassium acetate, and 15 grams of powdered potassium chloride, and stir to hasten solution. Add 15 cc. of 95 per cent. alcohol and rub the side of the beaker vigorously with

[1] Windisch: *Z. Nahr. Genussm.*, **1905**, 70.

[2] Halenke and Moslinger: *Z. anal. Chem.*, **1895**, 263.

a glass rod for about 1 minute to start crystallization. Let stand at least 15 hours at room temperature; decant the liquid from the separated acid potassium tartrate as rapidly as possible on a Gooch crucible prepared with a thin film of asbestos, transferring no more of the precipitate to the crucible than necessary. Wash the precipitate and filter three times with a small amount of a mixture of 15 grams of potassium chloride, 20 cc. of 95 per cent. alcohol, and 100 cc. of water, using not more than 20 cc. of the wash solution in all. Transfer the asbestos film and precipitate to the beaker in which the precipitation took place, wash out the crucible with hot water, add about 50 cc. of hot water, heat to boiling, and titrate the hot solution with tenth-normal sodium hydroxide, using phenolphthalein as indicator. Increase the number of cubic centimeters of tenth-normal alkali employed by 1.5 cc. on account of the solubility of the precipitate. One cubic centimeter of tenth-normal alkali so consumed is equivalent to 0.0150 gram of tartaric acid.

Notes.—The above method depends on the precipitation of both the free and combined tartaric acid as acid potassium tartrate, which is then dissolved and titrated. In the presence of much free tartaric acid, however, the results are low, due, as pointed out by Hartmann and Eoff[1] to the reversible nature of the reaction, $KCl + H_2C_4H_4O_6 \rightleftharpoons HKC_4H_4O_6 + HCl$, by which a portion of the tartaric acid is not precipitated, the extent of the error being dependant on the amount of free tartaric acid present. This is the reason for the addition of potassium acetate, whereby potassium chloride and acetic acid are formed, thus offsetting the decomposing action of the hydrochloric acid. The temperature at which the reaction mixture is held during the 15 hours is also of great importance since more tartaric acid is precipitated the lower the temperature. They propose the following method[2] as giving much better results:

Preliminary Test.—Titrate 20 cc. of the well-shaken sample with $\frac{N}{10}$ sodium hydroxide until 2 drops of the solution give no red tint when mixed on a porcelain tile with several drops of neutral azo-litmin solution. If not more than 25 cc. of the

[1] *Bur. of Chem., Bull.* **162,** p. 71.

[2] See also *J. Assoc. Off. Agr. Chem.*, **1915,** 133.

standard alkali are required the wine may be used directly; if the amount exceeds 25 cc. the wine should be diluted with an equal volume of water.

Determination.—Neutralize 100 cc. of the wine (diluted or undiluted as the case may be) exactly with 2N sodium hydroxide, calculating the necessary amount from the preliminary titration. If the addition of the 2N alkali increases the volume of the solution more than 10 per cent., evaporate it to approximately 100 cc. Add pure tartaric acid, the amount to be added being calculated by the equation:

cc. 2N alkali for 100 cc. wine $\times 0.15 =$ grams tartaric acid required

Record the weight of tartaric acid added and after it is completely dissolved add 2 cc. of glacial acetic acid and 15 grams of potassium chloride. Stir until the potassium chloride is dissolved. The acid potassium tartrate usually begins to form before the potassium chloride is all dissolved but these two salts are readily distinguished from each other. Add 20 cc. of 95 per cent. alcohol, stir vigorously for 5 minutes, and let stand at least 15 hours at a temperature not exceeding 15°C. Then decant the solution through a Gooch crucible and wash with the mixture described in method (*a*), making three washings of 7 cc. each. With care the beaker, precipitate and crucible may be thoroughly freed from acetic acid with these washings. Transfer the precipitate and asbestos with hot water to the original beaker, add about 50 cc. of water, bring to a boil and titrate with $\frac{N}{10}$ sodium hydroxide and phenolphthalein.

The grams of tartaric acid in 100 cc. is found from the following:

$$X = [(A + 1.5) \times 0.015] - T$$

where A is the cc. of $\frac{N}{10}$ alkali used for titration, and T is the grams of tartaric acid added.

(*b*) **Cream of Tartar.**—Ignite 25 or 50 cc. of the wine cautiously to a white ash and determine the alkalinity of the soluble ash as described on page 19. Calculate as potassium acid tartrate, $HKC_4H_4O_6$.

(*c*) **Free Tartaric Acid.**—Add 25 cc. of $\frac{N}{10}$ hydrochloric acid to the ash of 25 or 50 cc. of wine, heat to incipient boiling and titrate with $\frac{N}{10}$ sodium hydroxide, using phenolphthalein as indicator. Multiply the number of cubic centimeters of standard acid consumed by 0.0150 to obtain the equivalent in grams of tartaric acid and subtract this (expressed as grams per 100 cc.) from the result obtained under (*a*) to obtain the free tartaric acid.

Notes.—Methods (*b*) and (*c*) are based on the assumption that by ignition the potassium acid tartrate is converted to an equivalent amount of potassium carbonate, while the free tartaric acid burns to volatile products. It is necessary in method (*c*) to determine the alkalinity of the total ash, since a small amount of the tartaric acid may be present as an alkaline earth tartrate which would leave an insoluble carbonate.

The relation of the different forms in which the tartaric acid may be present, as calculated from the titration, has been well summarized by Fresenius and Grünhut.[1] If A represents the corrected acidity of the acid potassium tartrate obtained in the determination of total tartaric acid (page 441),[2] G the alkalinity of the total ash and W the alkalinity of the water-soluble ash, all expressed as cubic centimeters of normal alkali per 100 cc. of the wine, then

(*a*) If A is greater than G,

Total tartaric acid	=	$0.15A$[3]
Cream of tartar	=	$0.1881W$[3]
Alkaline earth tartrates	=	$0.15\ (G - W)$
Free tartaric acid	=	$0.15\ (A - G)$

(*b*) If A is equal to or smaller than G, but greater than W,

Total tartaric acid	=	$0.15A$
Cream of tartar	=	$0.1881W$
Alkaline earth tartrates	=	$0.15\ (A - W)$
Free tartaric acid	=	0.0

[1] *Z. anal. Chem.*, **1899**, 477. (See also *J. Assoc. Off. Agr. Chem.*, **1915**, 132.)

[2] To the number of cubic centimeters of $\frac{N}{10}$ alkali used add 1.5 cc. and divide the whole by 10 to convert it to normal alkali.

[3] Molecular weight of tartaric acid = 150; of acid potassium tartrate = 188.1.

(*c*) If *A* is equal to or smaller than *W*,

Total tartaric acid	=	0.15*A*
Cream of tartar	=	0.1881*A*
Alkaline earth tartrates	=	0.0
Free tartaric acid	=	0.0

Glycerin.—(*a*) *Dry Wines.*—(With less than 2 grams of sugar in 100 cc.) Follow the method described for the determination of glycerin in Vinegar, page 369, using 100 cc. of the sample and making only one preliminary evaporation.

(*b*) *Sweet Wines.*—(With more than 5 grams of extract or 2 grams of sugar in 100 cc.) Heat 100 cc. of the wine to boiling in a flask and treat it with successive small portions of milk of lime until it becomes first darker and then lighter in color and has an odor of alkali. When cool add 200 cc. of 95 per cent. alcohol, allow the precipitate to settle, filter and wash with alcohol. Evaporate the filtrate and proceed as described on page 369 under Vinegar.

Notes.—The above method is practically the official German method except that the determination of the extracted glycerin by oxidation with potassium bichromate has been substituted for the admittedly inexact weighing of the crude glycerin. The method is tedious in the extreme and in the case of plastered wines or those containing much sugar yields results which are distinctly too high. On account of the importance of the determination many other methods have been proposed to avoid these difficulties. Of these one of the simplest and most satisfactory is to distil the glycerin under reduced pressure.[1] The method is as follows:

Evaporate 30 cc. of wine to about 5 cc. on a sand-bath and rub thoroughly with 15 grams of plaster of Paris. The powder thus obtained is extracted for 6 hours in a Soxhlet extractor with hot absolute alcohol and the extract, after the addition of 10–20 cc. of water, heated until the alcohol is entirely expelled. Transfer the residue to a 100 cc. retort, the tubulus of which is closed by a good cork, through which passes a short glass rod well lubricated with vaseline. The neck of the retort passes into a short condenser which is connected with a small, strong Erlen-

[1] Von Törring: *Z. anal. Chem.*, **1889**, 363; Suhr: *Arch. f. Hyg.*, **1892**, 305; Partheil: *Z. anal. Chem.*, **1895**, 389.

meyer flask, from which a tube leads to a manometer and an efficient pump (Fig. 60). Heat the retort in a small sheet-iron air-bath (at ordinary pressure) at 150°–170°C. until the water is driven off, then connect with the pump and raise the temperature to 190°–210°C. After the distillation, which usually requires about an hour, remove the flame, cool the air-bath by removing the cover, and disconnect the pump. In order to recover the glycerin which has condensed in the neck of the retort, add 3–5 cc. of water through the cork, again insert the glass rod, and distil, without the pump and with the water drawn from the condenser. In the distillate determine the glycerin by the Hehner method as described on page 371.

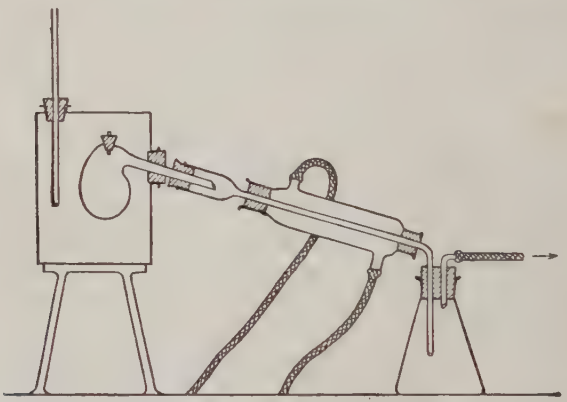


FIG. 60.—Apparatus for glycerin determination.

Still other methods which find some use are those of Trillat,[1] depending on solution of the glycerin in ethyl acetate, and of Zeisel and Fanto,[2] in which the glycerin is converted into iso-propyl iodide by heating with hydriodic acid and weighed as silver iodide. For these methods reference should be made to the original papers.

Reducing Sugars.—Measure 100 cc. of wine into a porcelain dish, neutralize exactly with an approximately normal sodium hydroxide solution and evaporate on the water-bath to about 25

[1] Trillat: *Compt. rend.*, **1902,** 903; Billon: *Rev. intern. falsif.*, **1906,** 57; *Z. anal. Chem.*, **1909,** 388.

[2] *Z. Nahr. Genussm.*, **1904,** 292; **1905,** 115.

cc. Wash into a 100 cc. flask, add sufficient neutral lead acetate to clarify, fill to the mark, mix thoroughly and filter through a dry filter. Remove the lead from the filtrate with potassium oxalate as described on page 257, filter, and in an aliquot part of the filtrate determine the reducing sugar by the Munson and Walker method, page 237. Calculate the result as invert sugar.

Notes.—The aliquot portion taken for the determination should be such that the limits stated on page 239 are not exceeded. This may be calculated approximately by assuming 2 grams per 100 cc. as the sugar-free extract of normal wine.

It will probably be found advisable to re-dissolve the cuprous oxide and determine it by one of the methods given on page 240, *et seq.* Cane sugar, which is occasionally present, may be determined if desired by determining also the reducing sugar after inversion. (See page 245.)

Polarization.—Polarize a part of the lead-free filtrate obtained in the preceding method in a 200 mm. tube. Express the result as the polarization of the undiluted wine in terms of the Ventzke scale. (See page 253.)

Note.—The polarization of normal wine is ordinarily to the left. If a right-handed polarization is found it may be due to unfermented cane sugar or commercial glucose. In the former case the polarization will be to the left after inversion, in the latter it will probably still be to the right. For the further examination of such a sample to detect the presence of the unfermented constituents of commercial glucose consult Bull. 107 of the Bureau of Chemistry, page 87, or Allen's Commercial Organic Analysis, 4th Ed., Vol. I, page 173.

Potassium Sulphate.—Acidify 50 or 100 cc. of the wine with hydrochloric acid, heat to boiling and precipitate with a slight excess of hot barium chloride solution. Let stand for half an hour, filter, wash and ignite the barium sulphate in the usual manner. Calculate as potassium sulphate.

Note.—There is always a small amount of potassium sulphate present in grape juice, and a small quantity of sulphuric acid results from the oxidation of part of the sulphur dioxide used in sulphuring the casks, so that the wine will contain on an average about 0.1 per cent., calculated as potassium sulphate. Any

excess over 0.2 per cent. is usually accepted as evidence of plastering.

Tannin.—Dealcoholize 25 or 50 cc. of wine by evaporation and dilute with water to the original volume. On 10 cc. of the solution determine the oxidizable material as described under Cloves, page 352. Call the number of cubic centimeters of permanganate used *a*.

Treat 10 cc. of the dealcoholized wine, prepared as above, with bone-black[1] for 15 minutes; filter and wash the bone-black thoroughly with water. Add 750 cc. of water and titrate as before. Call the number of cubic centimeters of permanganate used *b*.

Then $a - b$ = the cubic centimeters of permanganate required to oxidize the tannin and coloring matter in 10 cc. of wine.

The tannin equivalent of permanganate usually taken for wine is 0.004157, that being the value found by Neubauer for gall tannin, that is, 1 cc. of $\frac{N}{10}$ permanganate = 0.004157 gram of tannin. The amount of permanganate used up by the coloring matter is so slight that it may be neglected.

Note.—In addition to alcohol the wine contains also certain non-volatile substances which are capable of reducing permanganate. The alcohol is removed by evaporation and the tannin and coloring matter are held back by the bone-black, while the other oxidizable substances are determined in the second titration.

Sulphurous Acid and Sulphites.—Use 50 or 100 cc. of the wine and carry out the determination as described on page 100 under Preservatives.

Other Preservatives.—The preservatives other than sulphurous acid which may occasionally be found include salicylic acid, boric acid, sodium benzoate and saccharin. In Europe the use has been reported also of cinnamic acid, abrastol and hexamethylene-tetramine.

Salicylic Acid.—Since some genuine wines have been reported as giving tests for a small quantity of salicylic acid the precaution should be taken of not employing too large a sample

[1] Extract finely pulverized bone-black with hydrochloric acid and wash with distilled water until the acid is entirely removed. The bone-black is kept covered with water.

Use 50 cc. and carry out the test as described on page 95. If a positive test is obtained and doubt exists as to its being due to added salicylic acid resort may be had to the quantitative determination.

Benzoic Acid and Benzoates.—Follow out the method given on page 90.

Boric Acid.—Boric acid is a normal constituent of wine, so that tests, to have any significance, should be quantitative. To 100 cc. of wine add sodium hydroxide until slightly alkaline, ignite to a white ash and proceed as directed on page 99.

Saccharin.—See page 103.

Abrastol.—This substance, known also as *asaprol*, is the calcium salt of β-naphtholsulphonic acid. It has been reported used as a preservative in wine and also to take the place of gypsum in plastering. To detect it[1] boil 200 cc. of the wine with 8 cc. of concentrated hydrochloric acid for an hour under a reflux condenser. β-naphthol splits off and can be detected by extracting with 10 cc. of chloroform and heating the extract a few minutes with a little alcohol and several pieces of potassium hydroxide. In the presence of β-naphthol a deep blue color forms, rapidly changing to green and yellow. If only a small amount of abrastol is present the solution may be greenish and only the pieces of alkali colored blue.[2]

Cinnamic Acid.—This may be detected by shaking out a portion of the ether solution obtained in the test for benzoic acid (page 90) with 5 cc. of dilute $(\frac{N}{3})$ sodium hydroxide. Heat on the water-bath with a few drops of a 5 per cent. potassium permanganate solution. With 0.01 milligram or more of cinnamic acid a distinct odor of benzaldehyde will be noticed.[3] (See also page 91.)

Hexamethylenetetramine.—The use of this, under the name *Urotropin*, has been recommended for removing sulphites from wine. It may be detected by distilling the wine with phosphoric or sulphuric acid, by which the hexamethylenetetramine is decomposed into ammonia and formaldehyde. The latter will

[1] Sanglé-Ferriere: *Compt. rend.*, **1893**, 796; *Rev. intern. falsif.*, **1894**, 15.
[2] Wolff: *Pharm.-Ztg.*, **1895**, 44.
[3] von d. Heide and Jakob: *Z. Nahr. Genussm.*, **1910**, 145.

appear in the first portion of the distillate and may be identified by the tests given in Chapter III or the phenylhydrazine reaction described on page 125. This method, of course, does not distinguish the hexamethylenetetramine from formaldehyde itself. According to Rosenthaler and Ungerer[1] the most delicate test for hexamethylenetetramine itself is a 5 per cent. solution of mercuric chloride, which will give characteristic crystals with 1 part in 500,000. Treat white wines with the reagent directly, after acidifying with hydrochloric acid; treat red wines with solid basic lead acetate, filter, remove the excess of lead with sodium phosphate and apply the test.

Artificial Color.—Red wines are the only ones that need to be examined for artificial color, since with white wines practically the only additions made to change the color are such substances as caramel, chicory extract and possibly minute traces of one or two special coal-tar dyes. With red wines a general idea of the likelihood of artificial color being present may be gained by applying the following simple preliminary tests:

(*a*) *Basic Lead Acetate Test.*—Add to 25 cc. of wine 5 cc. of basic lead acetate solution, shake and note the color of the precipitate. Natural wines impart to the precipitate a gray, bluish-green or green color; certain vegetable colors, like poke berry or bilberry extract, give a purple color. Add 5 cc. more of the basic lead acetate solution, heat the mixture and filter. Natural wines will, in general, give a very pale or nearly colorless filtrate; if the filtrate is colored distinctly red and yields a red color to amyl alcohol when shaken with it, an artificial color may be present.

(*b*) *Mercuric Oxide Test.*[2]—Add to 10 cc. of the wine 0.2 gram of yellow mercuric oxide, shake violently for at least a minute and after settling filter through a double, wet filter. If the filtrate is not perfectly clear repeat the test, warming the mixture before shaking.

A clear, colored filtrate indicates the presence of a coal-tar color, but on the other hand, a colorless filtrate should not be taken as necessarily implying the absence of such color, since

[1] *Pharm. Zentr.*, **1913**, 1153.

[2] Cazeneuve: *Compt. rend.*, **1886**, 52; Spaeth: *Z. Nahr. Genussm.*, **1899**, 633.

Erythrosin and Eosin, for example, are absorbed by the mercuric oxide. Other colors, as Orange I and Safranin, are partly absorbed, so that if present in slight amount they might escape detection. Such colors as Bordeaux Red, Amaranth and Crocein Scarlet are readily shown by this test.

(*c*) *Formaldehyde Test.*[1]—To 50 cc. of wine add 1 cc. of formalin (40 per cent. formaldehyde) and 4 cc. of hydrochloric acid and heat on the water-bath until a precipitate forms. Then make slightly ammoniacal and continue the heating until the ammonia is expelled, cool and filter. Natural wines will, in general, give a colorless filtrate in this test, while the converse is true of artificially colored samples. Like the other simple tests, however, the results must be interpreted with some caution, since it would hardly be safe to assume without further testing in the case of a colorless filtrate that all artificial colors were absent, and further, it has been shown[2] that the test fails with a few very deeply colored genuine wines.

If the results of the preliminary tests indicate the possible presence of an artificial color a further careful study should be made by the methods outlined in Chapter III, especially the double dyeing method on wool and the examination of the color extracted by amyl alcohol from the acid and the ammoniacal solution.

The older text books give complicated procedures for the detection of many vegetable colors, but the reactions are in many cases inconclusive and require much experience for their proper interpretation.

Interpretation of Results

The examination of a wine may be made to determine whether it is true to name or contains any deleterious or forbidden substance, in a word, if it is free from adulteration; or the examination may be to determine the soundness, quality or capacity for improvement on keeping. An examination for the second purpose demands on the part of the analyst a wide experience with the class of wines in question, and in most cases a trained sense of taste is of as much assistance in forming a judgment as

[1] Jean and Frabot: *Ann. chim. anal.*, **1907**, 52.
[2] Astruc: *Ann. chim. anal.*, **1907**, 140.

chemical tests. For this reason, the discussion taken up here will necessarily be limited to the detection of adulterations, and for that matter, to certain comparatively gross forms of sophistication and only as they can be shown with some degree of certainty by chemical analysis.

In the customary wine analysis, then, the questions to be answered are such as: Has any improper treatment been employed, or forbidden substance added, during the manufacture of the wine? Is the wine of the character or type claimed by the label? Has any foreign substance, sugar, water, alcohol, etc., been added to the product or during the fermentation?

Analyses of Authentic Samples.—Since European countries were the originators of the modern wine industry and their products still command the highest reputation, it is only to be expected that most of the published analyses of wine are by French and German chemists. Based on these analyses, certain broad standards have been laid down for genuine wines produced by the fermentation of normal grape musts. Even with the extended study which has been given to the subject, however, these standards are by no means of universal application in European practice and when applied to American wines, which are the ones with which we are most concerned, they can be considered only general guides. This difference in the American product is due partly to the different varieties of the wine-grapes grown in this country and partly to different soil and climatic conditions. It is undoubtedly due also to somewhat less finished methods of manufacture, so that as American wine-makers gain experience there is less divergence to be expected between their product and the wines of Europe. This has already been noted in comparing analyses of American wines with those of an earlier period.[1]

A compilation of several thousand reported analyses of wines, chiefly of European origin, will be found in König: Chemie der menschlichen Nahrungs- und Genussmittel. A somewhat similar compilation, comprising, of course, a much more limited number of samples, has been made of American wines by Bigelow.[2] Neither of these will be found of the greatest help to the be-

[1] *Bur. of Chem., Bull.* **72**, p. 22.
[2] *Bur. of Chem., Bull.* **59.**

ginner in wine analysis, however; the first because there are so many analyses of wines from different sources that one is at a loss as to which to choose for representative figures, and the second because the analyses are taken from all parts of the country, made at different times by different analysts, and admittedly include some not of known purity.

For this reason it has seemed best to limit the analyses given here to the maximum, minimum and average figures found in Table LXXX. These are compiled from the analyses of American wines which received awards at the Paris exposition of 1900 and should therefore be typical of the highest grades of genuine American wines. Further, the analysis made was more thorough than in many other published analyses. The details of the analyses and description of the samples may be found in Bulletin 72 of the Bureau of Chemistry. It should perhaps be added that these are given simply to show the range of values to be expected in the various types of American wines, and are not at all to be considered as limiting values for the pure product.

Federal Standards.[1]—1. *Wine* is the product made by the normal alcoholic fermentation of the juice of sound, ripe grapes and the usual cellar treatment, and contains not less than 7 nor more than 16 per cent. of alcohol, by volume, and, in 100 cc. (20°C.), not more than one-tenth gram of sodium chloride nor more than two-tenths gram of potassium sulphate, and for red wine not more than fourteen-hundredths gram, and for white wine not more than twelve-hundredths gram of volatile acids produced by fermentation and calculated as acetic acid. *Red wine* is wine containing the red coloring matter of the skins of grapes. *White wine* is wine made from white grapes or the expressed fresh juice of other grapes.

2. *Dry wine* is wine in which the fermentation of the sugars is practically complete and which contains, in 100 cc. (20°C.), less than 1 gram of sugars, and for dry red wine not less than sixteen-hundredths gram of grape ash and not less than one and six-tenths grams of sugar-free grape solids, and for dry white wine not less than thirteen-hundredths gram of grape ash and not less than one and four-tenths grams of sugar-free grape solids.

[1] U. S. Dept. of Agr., Office of the Secretary, Circular 19.

TABLE LXXX.—ANALYSES OF AMERICAN WINES RECEIVING AWARDS AT PARIS, 1900
(Grams per 100 cc.)[1]

Determination	Sparkling wines			Dry white wines			Dry red wines			Sweet white wines			Sweet red wines		
	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.
pecific gravity 15.5°C	1.0169	0.9910	1.0045	0.9939	0.9901	0.9917	0.9969	0.9926	0.9943	1.0494	0.9908	1.0298	1.0522	1.0107	1.0276
lcohol	12.06	9.25	10.48	11.31	8.41	10.17	12.22 10.71	8.01	10.00	17.10	9.21	14.53	17.50	10.87 13.85	15.31
lycerin	0.7330	0.2301	0.4177	1.0119	0.5690	0.7019	0.9504	0.5341 0.5940	0.6355	0.7350 0.5924	0.0483 0.1316	0.3025	0.7460	0.2936	0.5089
ycerin-alcohol ratio				9.2:100	5.7:100	7.1:100	8.1:100	5.8:100	6.4:100						
xtract	8.56	1.78	5.40	2.51	1.55	1.99	3.22	1.77 2.02	2.57	19.35	2.83 5.71	13.80	19.71	7.57 10.69	13.52
sh	0.290 0.171	0.114	0.153	270	0.107 0.160	0.196	0.393	0.138	0.247	0.263	0.097	0.203	0.374	0.234	0.311
xtract-ash ratio	1:30.1 1:16.9	1:8.5	1:14.6	1:16.4 1:13.0	1:6.7	1:10.3	1:18.6 1:14.8	1:6.5	1:10.6	1:287 1:15.6	1:4.5 1:8.8	1:15.0	1:16.6	1:8.4	1:11.1
otal acids	0.783	0.601	0.658	0.715	0.433	0.586	0.901	0.454	0.649	0.805 0.534	0.160 0.201	0.412	0.826 0.546	0.397	0.502
xed acids	0.715	0.418	0.566	0.570	0.326	0.459	0.634	0.346	0.507	0.528	0.116	0.300	0.508	0.257	0.472
olatile acids	0.148	0.049	0.082	0.174	0.050	0.101	0.266	0.071	0.128	0.222 0.124	0.029 0.056	0.092	0.255 0.144	0.080	0.122
tal tartaric acid	0.357	0.163 0.205	0.271	0.352 0.273	0.059 0.119	0.189	0.252	0.083	0.163	0.296	0.038 0.067	0.142	0.145 0.102	0.025	0.078
ee tartaric acid	0.141	0.000	0.065	0.1785	0.0000	0.0677	0.0468 0.000	0.000	0.000	0.1436	0.000	0.0166	0.000	0.000	0.000
olatile acid-total acid ratio	1:14.50	1:4.07	1:9.21	1:9.88	1:383	1:6.21	1:7.90	1:3.38	1:5.71	1:9.47 1:6.69	1:2.96 1:3.53	1:4.93	1:5.78	1:3.24	1:4.39
arization, °V	−13.3 − 8.0	−1.8	−5.2	−1.1	−0.1	−0.5	−0.8	0.0	−0.4	−25.7 −14.2	0.0 −8.8	−11.8	−19.4	− 7.7 −15.6	−16.4
ducing sugars	5.228	0.023 2.390	3.409	0.328	0.051	0.134	0.280 0.214	0.045	0.146	16.91	1.76 3.71	11.30	16.96	3.24 7.66	10.26
otein	0.698 0.222	0.070	0.214	0.337 0.235	0.060 0.109	0.162	0.305	0.077	0.150	0.245	0.026 0.105	0.162	0.368 0.281	0.105	0.232
assium sulphate	0.128 0.047	0.015	0.033	0.130	0.026	0.087	0.133	0.016 0.045	0.070	0.088	0.007 0.021	0.044	0.062	0.024	0.048
osphoric acid	0.026	0.012	0.017	0.066	0.010	0.022	0.044	0.015	0.029	0.059	0.008	0.034	0.068	0.018 0.038	0.045
e sulphurous acid				0.0078	0.0002	0.0017									
al sulphurous acid	0.0456 0.0051	0.0010	0.0074	0.0163	0.0013	0.0063				0.0188 0.0052	0.0011	0.0045			
nin	0.066	0.009	0.035	0.0830 0.0641	0.0178	0.0391	0.3435	0.1388	0.2364	0.0662 0.0445	0.0213	0.0363	0.2207 0.1371	0.0516	0.0962

[1] In some cases the next to the extreme value is given also.

3. *Fortified dry wine* is dry wine to which brandy has been added but which conforms in all other particulars to the standard of dry wine.

4. *Sweet wine* is wine in which the alcoholic fermentation has been arrested and which contains, in 100 cc. (20°C.), not less than 1 gram of sugars, and for sweet red wine not less than sixteen-hundredths gram of grape ash, and for sweet white wine ot less than thirteen-hundredths gram of grape ash.

5. *Fortified sweet wine* is sweet wine to which wine spirits have been added. By act of Congress, "sweet wine" used for making fortified sweet wine and "wine spirits" used for such fortification are defined as follows (section 43, act of October 1, 1890, 26 Stat. 567, as amended by section 68, act of August 27, 1894, 28 Stat. 509, and further amended by act of Congress approved June 7, 1906): "That the wine spirits mentioned in section 42 of this act is the product resulting from the distillation of fermented grape juice to which water may have been added prior to, during or after fermentation, for the sole purpose of facilitating the fermentation and economical distillation thereof, and shall be held to include the products from grapes or their residues commonly known as grape brandy; and the pure sweet wine, which may be fortified free of tax, as provided in said section, is fermented grape juice only, and shall contain no other substance whatever introduced before, at the time of, or after fermentation, except as herein expressly provided; and such sweet wine shall contain not less than four per centum of saccharine matter, which saccharine strength may be determined by testing with Balling's saccharometer or must scale such sweet wine, after the evaporation of the spirits contained therein, and restoring the sample tested to original volume by addition of water; provided that the addition of pure boiled or condensed grape must or pure crystallized cane or beet sugar or pure anhydrous sugar to the pure grape juice aforesaid, or the fermented product of such grape juice prior to the fortification provided by this act for the sole purpose of perfecting sweet wine according to commercial standard, or the addition of water in such quantities only as may be necessary in the mechanical operation of grape conveyors, crushers, and pipes leading to fermenting tanks shall not be excluded by the definition of pure sweet wine aforesaid;

provided, however, that the cane or beet sugar, or pure anhydrous sugar, or water so used shall not in either case be in excess of ten per centum of the weight of the wine to be fortified under this act; and provided further, that the addition of water herein authorized shall be under such regulations and limitations as the Commissioner of Internal Revenue, with the approval of the Secretary of the Treasury, may from time to time prescribe; but in no case shall such wines to which water has been added be eligible for fortification under the provisions of this act where the same, after fermentation and before fortification, have an alcoholic strength of less than five per centum of their volume."

6. *Sparkling wine* is wine in which the after part of the fermentation is completed in the bottle, the sediment being disgorged and its place supplied by wine or sugar liquor, and which contains, in 100 cc. (20°C.), not less than twelve-hundredths gram of grape ash.

7. *Modified wine, ameliorated wine, corrected wine,* is the product made by the alcoholic fermentation, with the usual cellar treatment, of a mixture of the juice of sound, ripe grapes with sugar (sucrose), or a sirup containing not less than sixty-five per cent. of sugar (sucrose) and in quantity not more than enough to raise the alcoholic strength after fermentation to eleven per cent. by volume.

8. *Raisin wine* is the product made by the alcoholic fermentation of an infusion of dried or evaporated grapes, or of a mixture of such infusion of raisins with grape juice.

These standards have not, of course, the force of law and are to be regarded, as in other chapters where they have been quoted, simply as extreme values so fixed as to include practically all commercial goods honestly made with no attempt to defraud.

The official definition of wine, as given in Food Inspection Decision 156, is practically identical with the above definition and, since the Department of Agriculture is given authority by Congress to frame regulations for enforcing the Food and Drugs Act, thereby legally excludes all substances other than those present in the juice of fresh grapes or ordinarily added in the usual cellar treatment. Principally for the benefit of the wines made in Ohio and Missouri, however, certain additions are allowed

for the correction of natural defects due to conditions of soil and climate. (See page 434.)

Characteristics of Genuine Wine.—*Alcohol.*—The amount of alcohol in natural unfortified wines is usually between 4.5 and 10 grams per 100 cc., although distinctly higher and lower values have been reported. Since the process of fermentation does not yield over 14.5 grams, values much in excess of this may be considered evidence of added alcohol.

Glycerin.—The glycerin content of normal wines is usually placed at 0.4 to 1.0 gram per 100 cc., although results as low as 0.12 and as high as 1.4 have been reported. There is more variation in this respect in sweet than in dry wines.

Alcohol-Glycerin Ratio $\left(\frac{100 \times \text{Glycerin}}{\text{Alcohol}}\right)$.—Since the normal fermentation of sugars with yeast, under restricted conditions, produces a fairly definite proportion of glycerin to the amount of alcohol, this ratio is of much more importance than either alone in deciding whether alcohol has been added to the wine. The former German standard fixed the limits for this ratio between 7 and 14, but the lower limit has been reduced to 6. Even this is rather high for American wines, the minimum ratio in Table LXXX being 5.7, and many of the analyses in Bulletin 59 showing even lower ratios. Possibly 5.5 would be a fairer standard, although not many of the high-grade domestic wines made at present would fall below the European standard of 6.

Extract.—As in the case of other fermented products in which sugar is the principal substance changed during the fermentation, the sugar-free extract in much more nearly constant than the total extract (see also under Vinegar, page 373), and lends itself much better to the fixing of a minimum standard. The amount of potassium sulphate in plastered wines should also be subtracted. Since wines contain normally a small amount of both sugar and potassium sulphate, a common formula in European practice for determining the "reduced extract," as it is called, is

$$E - (S - 0.1) - (K - 0.1)$$

where E is the grams of extract, S the sugar and K the potassium sulphate per 100 cc. of wine. The reduced extract for white wines usually varies between 1.5 and 2.6 grams, for red wines

between 1.8 and 3.0 grams. The amount of extract decreases slightly as the wine grows older, but will seldom fall below 1.5 grams per 100 cc. Compare also the standards for sugar-free extract given on page 453.

Ash.—The extreme limits which have been suggested for the ash of all wines are 0.11 to 0.44 gram per 100 cc. These extremes are not often met and fairer limits for usual practice would be set at 0.14 to 0.35 gram. Since these standards are stated in terms of "grape ash," any excess of potassium sulphate over 0.1 gram should be deducted.

Alcohol-Extract Ratio $\left(\frac{\text{Alcohol}}{\text{Extract}}\right)$.—French authorities place the maximum values for this ratio at 4.5 for genuine red wine and at 6.5 for white wines. Higher values than these are considered evidence of added alcohol. The figures are best taken in connection with the Glycerin-Alcohol ratio.

Acidity.—The total acidity, expressed as tartaric acid, usually lies between 0.5 and 0.9 gram per 100 cc., although values of 0.3 and 1.7 have been found.

The volatile acids are more a measure of the care taken in the fermentation and handling of the wine than a criterion of freedom from adulteration. The maximum limit adopted in European countries is 0.12 gram per 100 cc. for normal wines, and values over 0.15 gram are held to indicate an unsound wine. Conditions in this country are such that the values adopted might well be somewhat higher, although these limits are practically those given in the standards on page 453.

Of greater value for the detection of adulteration is the amount of free tartaric acid and its relation to the fixed acids since normal wine from ripe grapes contains no free tartaric acid, and only a slight amount is found even in wine from unripe grapes. Kunz[1] reports values of 0–0.105 gram per 100 cc. (average = 0.025) for free tartaric acid, while other observers have found from 0.1 to 0.3 gram in wines from unripe grapes. The German Commission makes the general rule that the free tartaric acid should not exceed one-sixth of the fixed acid provided that the amount of total free acids is not more than

[1] *Z. Nahr. Genussm.*, **1901**, 673.

0.8 gram per 100 cc. Natural wines with higher total acid frequently contain more free tartaric acid.[1]

Detection of Specific Adulterations.—(*a*) *Added Alcohol.*—As stated on page 457, the presence of more than 14.5 grams of alcohol per 100 cc. is direct evidence of added alcohol, an addition which is allowable in the case of fortified sweet wines (see page 455). With wines which contain a less amount than 14.5 grams the safest guide is the ratio between the alcohol and glycerin. Wines which contain less than 6 grams of glycerin to 100 of alcohol are generally regarded as having added spirit. On the other hand, wines with very low glycerin content, corresponding to 6 grams or less of alcohol (less than 0.36 gram of glycerin), have probably been made by arresting the fermentation of the must through the addition of alcohol.

A criterion of the presence of added alcohol which is frequently quoted is the alcohol-extract ratio (page 458). A value for the ratio; $\frac{\text{alcohol}}{\text{reduced extract}}$, greater than 4.5 for red wines or 6.5 for white wines is considered by French authorities as indicative of added alcohol. The "natural alcohol" is calculated in such a case by multiplying the reduced extract by 4.5 or 6.5 as the case may be.

(*b*) *Watering.*—The problem of showing added water in wine is almost analogous to the same question in Milk (page 132). The same suggestion has been made, that is, to show the presence of the water by impurities which it may contain, chiefly by its content of nitrates, but further observations have shown that such methods are not reliable. The only practical method of determining whether water has been added is by comparison with a similar wine of known purity to show whether the characteristic constants, ash, extract, acids and alcohol, have all been lowered, and in approximately the same proportion.

For French wines the relation between the acids and the alcohol is considered important for showing watering. According to Gautier,[2] in normal wine the amount of alcohol varies inversely as the free acids, the sum of the two being nearly a constant. The usual form adopted for expressing this relation

[1] Kulisch: *Chem.-Ztg.*, **1908,** 1105.

[2] Traité sur la Sophistication et l'Analyse des Vins.

is to add to the per cent. of alcohol by volume the total acid, calculated as grams of sulphuric acid per liter. A value for the figure thus obtained below 13 for red wines or below 12 for white wines is considered to indicate added water.

If the wine has been shown according to (*a*) to contain added alcohol, the value used in the above calculation should not be the per cent. of alcohol as found but the per cent. by volume corresponding to the "natural alcohol," calculated as previously described.

(*c*) *Plastering.*—Additions of gypsum in such quantity as to raise the sulphuric acid content of the wine, expressed as potassium sulphate, over 0.2 gram per 100 cc. are definitely forbidden by the wine laws of European countries and the same limit is incorporated in the Federal standards (page 453). Smaller additions than this are best shown in the increased sulphuric acid in the ash. Natural wines do not ordinarily contain more than 0.01 to 0.014 gram of sulphuric acid (SO_3), corresponding to 3.8 to 25 per cent. (average, 10.5 per cent.) of the ash, while the ash of plastered wines frequently contains 40 per cent. or more. There is also a corresponding decrease in the alkalinity and carbonic-acid content of the ash.[1]

(*d*) *Sugared Wines.*—The European wine-producing countries are inclined to look with disfavor upon the addition of sugar, either dry or as a solution, to wine. Under exceptional circumstances the addition of small quantities of pure dry sugar may be permitted if necessary to correct defects in the product. An extension of this practice is the addition to the must of a sugar solution or sugar syrup (*gallization*). Such products, however, can not be sold under the name wine without qualification. (See in this connection, Ameliorated Wine, page 456, and Food Inspection Decision 156, page 434.)

1. *Addition of Dry Sugar* (*Sucrose*).—If the added sugar has not been entirely fermented, a qualitative test for its presence will be sufficient in case of a positive result to show that sugar has been added, since natural wines contain no sucrose. A test frequently used to show this is that of Rothenfusser.[2]

[1] Carpentiere: *Z. Nahr. Genussm.*, **1912**, 42.

[2] *Z. Nahr. Genussm.*, **1909**, 135. Heat 25 cc. of wine on the water-bath to 85°–90°C. Add an equal volume of a freshly prepared mixture of 2 parts

If the sugar added was anhydrous grape sugar (glucose) and it has not all been fermented, its presence will ordinarily be shown by the determin tion of reducing sugar and polarization. Any residual unfermented sugar in a natural wine is mainly levulose, since the dextrose is more readily fermented. In fresh musts the ratio of dextrose to levulose has been observed to be 100 : 77–84; after a week's fermentation, however, the wine contained one and one-half to six times as much levulose as dextrose.[1] In natural wine, then, the polarization would be to the left, while the presence of an appreciable amount of anhydrous grape sugar would cause it to be to the right.

The addition of commercial invert sugar is much harder to detect, but even here, if the added sugar has not completely fermented it will be found that in natural wines the polarization is more strongly levo-rotatory as compared with the total reducing sugars, than would be the case with invert sugar itself, the difference being due again to the preponderance of levulose in the genuine product.

If the added sugar has been completely fermented, a clue to the addition may still be gained from the ratio of the acids to the alcohol, since the musts which are low in sugar, and hence would yield little alcohol if no sugar were added, are usually high in acidity. According to Windisch[2] an alcohol content of more than 9 grams per 100 cc. together with over 0.9 gram of total acids is suspicious of added dry sugar.

French authorities frequently calculate the sugar present in the original must in order to determine whether sugar has been added. This is taken as the sum of the sugar present in the wine, and twice the alcohol (since 100 parts of dextrose yield by fer-

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of lead acetate (500 grams in 1200 cc. water) and 1 part of ammonia (sp. gr. 0.94), shake violently for 30 seconds and filter. Mix 3 cc. of the clear, colorless filtrate with an equal volume of diphenylamine reagent (10 cc. of 10 per cent. alcoholic diphenylamine solution, 25 cc. of acetic acid, and 65 cc. of concentrated hydrochloric acid), heat 10 minutes in boiling water and note the appearance of a blue color. At the same time heat another portion of the filtrate in the same bath with an equal volume of Fehling's solution. If no reduction is observed, and the diphenylamine test shows a blue color, sucrose is present.

[1] König and Karsch: *Z. anal. Chem.*, **1895**, 3.

[2] Die chemische Untersuchung und Beurtheilung des Weines.

mentation approximately 50 parts of alcohol), both expressed in grams per 100 cc. Since it has been determined by many analyses that for French musts the sugar content never exceeds 32.5 grams per 100 cc., any excess of the calculated sugar over 32.5 grams is taken as indicating an addition of either sugar or alcohol.

2. *Addition of a Sugar Solution.*—If a portion of the added sugar remains still unfermented it may be detected as mentioned under (1). With thoroughly fermented wines, however, the sugaring must be detected by the lowering of some important values or changing of known ratios. The alcohol content is raised, while on the other hand the per cent. of acids, extract, ash and nitrogen are lowered. This decrease is not necessarily proportional to the dilution by the sugar solution, for according to Kulisch[1] new constituents of the extract, especially glycerin, are formed by the fermentation of the sugar. It is especially noticeable, however, in the ash, nitrogen and phosphoric acid.

The Swiss "Lebensmittelbuch"[2] fixes the following minimum limits for fixed acids and "extract rest" (see page 463) for natural, unsugared wines for each per cent. of alcohol.

Alcohol, per cent. by volume	Fixed acids, grams in 100 cc.	Extract rest, grams in 100 cc.	
		Red wines	White wines
7	0.66	1.05	0.85
8	0.57	1.10	0.90
9	0.50	1.15	0.95
10	0.45	1.20	1.00
11	0.41	1.25	1.05
12	0.38	1.30	1.10
13	0.36	1.35	1.15

The phosphoric acid (P_2O_5) should not be less than 0.008 gram in 100 cc.

(*e*) *Preservatives.—Sulphurous Acid.*—The only preservative tolerated in wine is sulphurous acid. For the detection of other preservatives see pages 448 to 450, noting the occasional presence of small quantities of some of them naturally in wines. The

[1] *Arb. a. d. Kais. Ges.-Amte.*, **1910, 1.**

[2] Ed. 3, p. 38.

form in which sulphurous acid is supposed to be added is that of the sulphur dioxide itself, as produced ordinarily by burning sulphur, rather than in the form of sulphites, and the amount which is allowed is not to exceed 350 milligrams per liter, of total sulphurous acid, of which not more than 70 milligrams shall be in the free state.[1] In other countries the amount allowed is frequently less, 200 milligrams per liter and 50, 20, or even 16 milligrams of the free acid, being variously set as the limits.[2]

(*f*) *Pomace Wine.*—The following table[3] illustrates the difference in composition between a genuine wine and the so-called pomace wines obtained by extracting the marc or pomace several successive times with sugar solutions and fermenting the products.

TABLE LXXXI.—COMPOSITION OF POMACE WINE

(Grams per 100 cc.)

Sample	Extract	Free acids	Cream of tartar	Tannin	Nitrogen	Ash	Potash (K_2O)	Lime (CaO)	Phosphoric acid (P_2O_5)
Genuine wine....	2.11	0.78	0.388	0.0122	0.0341	0.222	0.1086	0.0094	0.0205
1st Pomace wine.	1.63	0.49	0.273	0.0165	0.0107	0.217	0.1011	0.0098	0.0109
2nd Pomace wine.	1.22	0.39	0.203	0.0288	0.0025	0.162	0.0846	0.0111	0.0038
3rd Pomace wine.	0.91	0.34	0.158	0.0273	0.0022	0.138	0.0618	0.0131	0.0030
4th Pomace wine.	0.88	0.33	0.063	0.0316	0.0003	0.100	0.0397	0.0160	0.0020

It will be observed that the pomace wines are low in extract, acids, nitrogen and phosphoric acid, but high in tannin. The ash, especially the potash and lime salts, is usually high, but occasionally may be found very low, hence is not so satisfactory as an indicator.

The ratio of the extract to the ash (see page 453) is frequently higher than 10:1 and even as high as 10:2.

The "undetermined extract" or "extract rest," as it is sometimes called, that is, the extract minus the sum of the fixed acids, glycerin and ash, according to Fresenius and Grünhut[4] is generally below the normal value for wine, 0.35 gram per 100 cc. When it is higher the increase is usually to be attributed to an abnormally high content of tannin. For this reason it has been

[1] Food Inspection Decision 76.

[2] Mastbaum: *Chem.-Ztg.*, **1908**, 427.

[3] Weigert: *Mitt. Versuchs. Klosterneuberg*, **1888.**

[4] *Z. anal. Chem.*, **1898**, 472.

suggested[1] to subtract from the extract five times the per cent. of tannin. With natural wines the difference should not be below 1.5.

Fresenius and Grünhut[2] state further that more than 0.03 gram of tannin per 100 cc. indicates usually either a pomace wine or the addition of tannin. It is true also that pomace wines have been found with much lower tannin content than this.

It has been claimed also that normal white wines contain about 0.1 gram per 100 cc. of tartaric acid combined with alkaline earths, while in pomace wines this value is either extremely low or entirely lacking. This result, however, should be interpreted with considerable caution on account of the variations it may possibly show through excessive sulphuring or by the possible direct addition of organic acids.

The glycerin-alcohol ratio in pomace wines is generally higher than 7 : 100 and frequently reaches 10 : 100.

The nitrogen content of natural wines is seldom under 0.007 gram per 100 cc., varying ordinarily between this value and 0.009, while for pomace wines the percentage present is very much lower.

The content of tartaric acid is naturally of great importance, the pomace wines usually containing distinctly less of this characteristic constituent. In the case of the United States vs. 60 Barrels of Wine (Notice of Judgment 3529), the contention was successfully made on behalf of the government that a true Ohio claret wine cannot possibly contain less than 0.2 per cent. of total tartaric acid, that the amount present is usually 0.3 per cent., and may be as high as 0.5 per cent. The wine in question, which was adjudged to be a pomace wine, contained 0.05 per cent. of total tartaric acid.

(*g*) *Imitation Wine.*—Entirely artificial wines, in whose preparation no grape juice has been used, may be found, although they are not so common as those in which a pomace or base wine has been so manipulated as to imitate a natural wine. These wines may be identified by discrepancies in the relation of the constituents ordinarily determined, since it would be extremely difficult, if not impossible, to prepare a mixture

[1] Barth: *Z. Nahr. Genussm.*, **1899**, 106.

[2] *Loc. cit.*

which should imitate a true wine in all its characteristics, including that of taste. Further, such a wine would be detected in most cases by the absence of the characteristic constituents, lecithin and inosite, which are almost invariably present in natural wine. Lecithin is present in amounts varying from 0.05 to 0.10 gram per 100 cc. For its detection and estimation see Leach: Food Analysis, 3d Ed., pages 265 and 349. To detect inosite, add to 200 cc. of wine, 20 cc. of basic lead acetate solution and 2–3 cc. of an alcoholic solution of tannin and filter. Remove the excess of lead with hydrogen sulphide, filter, decolorize by bone-black (see page 448) and concentrate to 10 cc. on the water-bath. Test a portion by Scheur's test,[1] depending upon the oxidation of inosite with nitric acid to colored oxyquinone derivatives, as follows:

Treat a few cubic centimeters of the concentrated solution with a little nitric acid and evaporate upon the water-bath almost to dryness; add a little ammonia and barium chloride solution and again evaporate. A rose-red color will develop in the presence of 0.5 milligram or more of inosite.

Adulterated Samples.—For further study in the interpretation of a wine analysis there have been gathered in Table LXXXII analyses of various adulterated samples which have been prosecuted successfully under the Food and Drugs Act. In most of the cases the defendants pleaded guilty, which is as good evidence as needed of the correctness of the analyst's judgment of the sample. A comparison of the values in the table with those given in Bulletin 72, or the average values in Table LXXX, will be found most helpful.

The description of the samples follows:

1. This product was labeled: "Select Riesling Wine, Special Vintage, ..." Misbranding was charged on the ground that the label would lead the purchaser to believe that the product was Riesling wine of a select quality, when as a matter of fact the analysis showed it to be a compound of wine and a fermented solution of commercial dextrose, otherwise known as starch sugar.

2. The principal label in this case read: "Special Queen of Lake Erie Ohio Scuppernong Wine . . . . ." The product

[1] *Ann.*, **1852**, 375.

was also "Guaranteed not to be adulterated or misbranded within the meaning of the National Food Law." Adulteration was alleged in this case for the reason that the product was not a true wine made from Scuppernong grapes, but was a mixture of pomace wine and a wine made from grapes other than Scuppernong grapes, and contained very little, if any, genuine Scuppernong wine. The question of the actual presence of Scuppernong wine was of course decided largely by the taste and general appearance of the wine. The analysis is cited here because it is a typical mixture of a pomace with a sweet wine.

3. In distinction from the preceding analysis, this illustrates the adulteration of a *dry* wine with a wine prepared from pomace. The package bore the label: "Ohio Catawba Wine . . . Special," but the conclusion drawn from the analysis was that the product was not genuine Catawba wine but was prepared in whole or in part from pomace.

In all of these cases cited the defendants pleaded *nolo contendere* and a fine was imposed by the court.

4. "Select Scuppernong Wine." From the analysis it was found that this sample was artificially prepared to resemble Scuppernong wine. Adulteration of the product was alleged in the information because of the substitution of a mixture containing sugar, water, flavor, and the juice of grapes other than Scuppernong wholly or in part for the genuine article. Note especially the proportion of tartaric acid and sucrose.

5. This sample is somewhat different from the others tabulated in that it was represented to be a genuine French champagne of high quality, "Extra Dry Superior Quality" appearing on the neck label, and "Sparkling Wine Extra Dry Les Etoiles D'Or" on the body label of the bottle. The product was held to be adulterated in that an imitation champagne of domestic origin, made in part from pomace wine and artificially carbonated, had been substituted in part for genuine sparkling wine champagne. The defendants pleaded guilty and a fine was imposed. Compare the analysis carefully with the figures given for sparkling wines in Table LXXX.

6. On the cases containing the wine appeared the statement: "Sauterne Extra Dry," and on the bottles the labels read: "Sparkling Carbonated Wine Extra Dry Sauterne Type . . . ."

By comparison with Table LXXX it is seen at once that this is not of the type of Sauterne, which is a dry white wine, and from the reducing sugar content it certainly is not extra dry. An indictment was successfully brought on the grounds that a domestic white wine, artificially carbonated and not extra dry, had been substituted entirely for genuine sauterne wine.

7. The analysis given for this sample is not so extensive as some of the others, but enough is given to bear out the contention of the chemist that the product, labeled "Old Bass Island Ohio Port," was not port wine but an imitation port wine or an unfinished wine insufficiently fortified with alcohol (see figures for red sweet wines in Table LXXX, and analyses of American port wine in Bulletins 59 and 72 of the Bureau of Chemistry). The defendant company pleaded guilty to the charge and was fined $100 and costs.

8. The final sample of the table bore the label: "Ohio Golden Eagle Sauterne Wine." The analysis shows, however, that the sample does not correspond at all to a dry white wine, but indicates the presence of a considerable proportion of a pomace wine, sweetened and flavored. The makers of the product did not contest the case but paid the fine imposed.

Whiskey

As was stated in the opening paragraphs of this chapter, in order to produce a beverage containing a greater amount of alcohol than is possible in a fermented liquor as beer or wine, resort must be had to distillation. The distilled liquors thus produced vary greatly in their flavor and in their general characteristics, depending upon the methods by which they are made and the raw materials used. Possibly the most logical one to take as typical would be *brandy*, this being obtained by the distillation of wine or wine residues, which have just been discussed. Brandy is, moreover, probably the highest grade of distilled liquor produced, being made from what is itself the purest fermented product, wine. In this country, however, a more characteristic product is that made by distilling a fermented grain mash, practically by distilling beer, and known as *whiskey*. For this reason it has been chosen as representative of this class of food products.

TABLE LXXXII.—ANALYSES OF ADULTERATED WINES
(Grams per 100 cc.)

Determination	I	II	III	IV	V	VI	VII	VIII
Specific gravity (15.6°/15.6°C.)	.....	1.0700	0.9901	1.0552	1.0021	1.0132	.....	1.0317
Alcohol (per cent. by volume)	12.95	12.91	13.07	12.50	11.77	11.63	7.15	13.12
Total solids (extract)	2.52	22.51	1.78	18.53	4.47	7.07	.....	12.58
Sugar-free solids	2.12	1.95	1.60	1.87	2.13	2.13	1.72	2.41
Glycerin	.....	.....	0.702	.....	.....	0.42	.....	.....
Reducing sugar	0.40	20.42	0.175	6.12	2.15	4.86	.....	10.07
Reducing sugar after inversion	0.40	.....	.....	.....	.....	.....	.....	.....
Sucrose	.....	0.14	.....	10.54	0.18	0.08	.....	.....
Total acid as tartaric	.....	0.634	0.533	0.596	0.713	0.628	0.495	0.630
Fixed acid as tartaric	.....	0.416	0.388	0.422	0.486	.....	0.456	0.436
Volatile acid as acetic	.....	0.174	0.116	0.139	0.181	0.104	0.031	0.155
Total tartaric acid	.....	0.144	0.165	0.114	0.227	.....	0.335	0.172
Free tartaric acid	.....	0.0	0.021	0 00	0.034	.....	0.137	0.00
Cream of tartar	.....	0.141	0.067	0.122	0.147	.....	0.180	0.210
Tartaric acid to alkaline earths	.....	0.030	0.090	0.010	0.075	.....	0.054	0.00
Tannin and coloring matter	.....	0.021	0.019	.....	0.009	.....	.....	.....
Polarization, direct, at 20°C. (°V.)	+3.5	−5.7	+0.5	.....	−3.0	−0.3	.....	−3.0
Polarization, invert, at 20°C. (°V.)	−3.5	−6.0	.....	.....	−4.0	−0.4	.....	−3.1
Ash	.....	0.122	0.146	0.172	0.194	0.196	0.110	0.192
Alkalinity water-soluble ash (cc. $\frac{N}{10}$ acid per 100 cc.)	.....	7.5	3.6	7.0	7.8	.....	.....	11.4
Alkalinity water-insoluble ash (cc. $\frac{N}{10}$ acid per 100 cc.)	.....	6.2	6.0	5.6	5.0	.....	.....	12.0
Total phosphoric acid (P_2O_5)	.....	0.0138	0.0136	.....	.....	0.0117	.....	.....
Chlorine (Cl)	.....	0.0153	0.0168	0.0253	0.0426	.....	.....	0.0256
Potassium oxide (K_2O)	.....	0.377	0.0349	0.0548	0.0454	.....	.....	.....
Sodium oxide (Na_2O)	.....	0.0101	0.0050	0.0139	0.0330	.....	.....	.....
Calcium oxide (CaO)	.....	0.0100	0.0134	.....	.....	.....	.....	.....
Magnesium oxide (MgO)	.....	0.0092	0.0063	.....	.....	.....	.....	.....
Sulphuric acid (SO_3)	.....	0.0100	0.0276	.....	.....	0.084	.....	.....
						(K_2SO_4)		

Definition.—Whiskey may be defined in a broad sense as a potable spirit obtained by distillation from a fermented grain mash composed of malt alone or of cereal grains saccharified by the diastase of malt.

It is understood in this definition by the use of the term "potable spirit" that the whiskey shall contain a certain proportion of the impurities, or as they are sometimes called, the "congeneric substances," which are produced with the alcohol during the fermentation and distillation and accompany it into the distillate. Indeed, it is mainly to these "impurities" that whiskey owes its characteristic flavor, since if the process of manufacture be so carried out that these are eliminated so far as possible the resulting product is a rectified spirit, containing over 95 per cent. of pure alcohol, and quite lacking in the taste and characteristics of what we ordinarily term whiskey.

This greater elimination of these secondary constituents from the distillate by the efficient modern or so-called "patent" stills has caused in the past much acrimonious discussion as to what the word whiskey shall actually include, some wishing to limit the term absolutely to the product of the simpler, older type of "pot still" which retains a large proportion of the congeneric substances. On the other hand, it has been contended that the word whiskey is applicable to all grain distillates, irrespective of the form of still employed. The latter view has been upheld by a British Royal Commission on potable spirits, which declined to limit the term to the product of the pot still, and in this country the word whiskey, without qualification, has been sanctioned for "All unmixed distilled spirits from grain, colored and flavored with harmless color and flavor, in the customary ways."[1]

Whiskey may be further distinguished in respect to the particular grain employed, as *rye whiskey*, made from a mixture of rye or barley malt and unmalted rye, and *Bourbon whiskey*, in the manufacture of which a large proportion of Indian corn (maize) is employed; or according to the country of origin as American, Scotch or Irish whiskey.

Manufacture.—Only the general outlines of the manufacturing processes can be taken up here and especially as they may affect the chemical composition of the finished product. For a

[1] Food Inspection Decision 113.

more detailed description of the methods and apparatus employed reference should be made to the works listed at the end of the chapter. The manufacturing processes group themselves naturally into three divisions: Mashing and Fermenting, Distilling, and Aging.

Mashing and Fermenting.—The finely ground grain, together oftentimes with a small proportion of malt, is heated with water until the starch is "pasted," then cooled, the main bulk of the malt added and the mixture kept at the proper temperature (140° to 150°F.) until the maximum amount of diastatic action has taken place, the object being to convert the starch to maltose as thoroughly as possible. Specially prepared yeast is now added and the fermentation allowed to continue for three to four days, when the resulting dilute alcoholic liquid or "beer" is ready for distillation.

Distilling.—It is common practice in this country to conduct the distillation in two operations. In the first, a so-called "beer still," acting either intermittently or continuously, is employed to concentrate the alcohol from the beer. In the continuous still, as the name indicates, the process goes on without interruption, the heated beer being added at the top of the still, through which steam is ascending, and the dealcoholized beer or "slop" being drawn off at the bottom. In the operation as conducted intermittently, each charge of the still is distilled as three fractions, the "heads," "middle run" and "tails." The heads and tails are commonly returned to the still and distilled with the next charge, so that the final products are the spent beer or slop, and the "high wines" containing about 60 to 70 per cent. of alcohol.

In both cases the product of the first distillation is subjected to a re-distillation in a steam-heated pot still, the middle portion of the distillate, when reduced to proof, constituting the crude or raw whiskey.

The distillation may be also carried on by the use of rectifying or column stills, which make use of the principle of fractionation by means of dephlegmators to obtain a more concentrated alcohol, nearly free from the secondary products. A partial rectification may also be secured by passing the distillate through tubes

containing charcoal, which removes a portion of the higher alcohols.

Aging.—The whiskey when first distilled is of a harsh, unpleasant flavor and is usually stored for some time in order that changes may take place in the secondary constituents, which tend to make the product more palatable. The effect of these changes upon the composition of the whiskey is discussed later. The storage of the whiskey during this period is conducted in oak barrels, much of the change in flavor and practically all of the color, being as a matter of fact due more to the wood of the barrel than to any chemical change in the whiskey itself. In order to increase the extent of the change, it is customary with American whiskey to char the interior of the barrels.

All of the processes which have been described are carried on under supervision of the Internal Revenue Bureau of the Federal Government, the materials and product being subject to government inspection from the time the grain is weighed for grinding and mashing until the aged whiskey is withdrawn from the market. Government officials are stationed at the distilleries for the purpose of enforcing this requirement. When the whiskey is placed in the government warehouse to be aged, it is stamped with a "warehouse stamp" which bears the date of inspection and the gauge. When the aged product is withdrawn, the tax is paid and another stamp attesting the fact affixed. This constitutes a "double stamped package."

For the further protection of the retail purchaser the whiskey after storage is allowed to be "bottled in bond." This amounts to a guaranty on the part of the government that the whiskey is at least four years old, of standard 100° proof, and that nothing has been added to it since it was distilled other than the water which may have been added to reduce it to 100° proof and what substances it may take up itself from the package in which it is kept in the warehouse. This is attested by a suitable seal issued by the Commissioner of Internal Revenue and placed over the cork before the bottle is allowed to be withdrawn for sale.

The outline of manufacturing processes given above has special reference to whiskey made in this country. Scotch and Irish whiskies are made by methods which are generally similar, although there is produced in those countries possibly a somewhat

larger proportion of liquor made by the cruder fire-heated pot still. The characteristic Scotch whiskies owe their peculiar smoky flavor and taste to the employment of peat as fuel in drying the malt.

General Composition.—The popular conception of whiskey as a solution of ethyl alcohol in water, containing an extremely minute quantity of substances which give it a taste and flavor, is as far wrong as the notion, so long held, that the injurious effects of whiskey are due mainly if not wholly to its content of "fusel oil." It is true that whiskey contains nearly half its volume of alcohol, but in addition it contains a number of other substances present in readily measurable quantities and so variable in amount that whiskey is in reality a very complex liquid. As regards the other prevalent belief, it is undoubtedly true that the toxic effects of fusel oil, in the proportion in which it is found in whiskey, have been greatly exaggerated, and it is also probably true that the preponderating agent in the whiskey which produces physiological effects is the ethyl alcohol.

These substances which are present along with the alcohol are in one sense impurities and were so termed on page 469, but from the standpoint of flavor they are valuable and necessary constituents. The case is in many ways analogous to maple sirup (see page 272), the principle constituent of which is sugar, but which owes its valued characteristics to small quantities of "impurities" which impart the special flavor for which it is prized. "They are the associated bodies which give the alcohol its special and valued characters, and to their production, modification, or elimination by age we owe the changes which spirits undergo during the process of maturing."[1]

The most important of these secondary constituents comprise: *Acids*, principally acetic and valeric, with traces of propionic and others; *esters*, chiefly ethyl acetate and valerate, from the ethyl alcohol, together with amyl acetate and valerate, derived from amyl alcohol; *furfural*, present in small but measurable quantities; *aldehydes*, principally acetaldehyde but, undoubtedly, including small amounts of other aldehydes resulting from the oxidation of the corresponding alcohols; *fusel-oil* or "*higher alcohols*," consisting mainly of amyl and iso-butyl alcohols,

[1] A. H. Allen: *Analyst*, **1891**, 102.

with smaller quantities of iso-propyl and normal propyl alcohols and traces of other alcohols, acids and ethers.

The proportion of the secondary constituents found naturally varies with the character of the materials and the exact methods of fermentation and distillation employed. Some of them, as the higher alcohols and the acids, are formed during the fermentation and hence are present in all whiskey in considerable amounts unless removed or decreased by the process of distillation. Others, of which the aldehydes and furfural are typical, are normally produced very largely during the distillation, either by the action of the live steam employed or by the charring of the materials when the still is directly fire-heated, hence will vary according to the exact apparatus and method used.

The changes which take place in the congeneric substances during the storage or aging of the whiskey, as they affect the interpretation of analyses, are discussed on page 488. It will suffice to say here that the popular theory that during the aging the higher alcohols are eliminated or so changed by oxidation that the whiskey loses its harsh and unpalatable character has been shown to be entirely incorrect. As a matter of fact, the proportion of the higher alcohols, as is true of practically all other constituents, is increased during the aging. The disagreeable taste of new whiskey has been ascribed by Schidrowitz[1] to the presence of pyrrol, as well as phenolic and sulphur compounds, which are either resinified or oxidized during the aging, so that they are not found in the matured product.

It is only comparatively recently that due consideration has been given to the changes produced in the composition of whiskey due to its being stored in charred barrels (see page 488). The storing in charred barrels is in effect a sort of rectification, since the action of the charcoal is not essentially different from the charcoal tubes or tanks used in some direct rectifying processes except that, in this case, we have to deal with the residual liquid rather than what has passed through the charcoal. This is important in itself, to say nothing of the tannin, color and solids taken up from the wood of the barrel.

[1] The Chemistry of Whiskey: *J. Soc. Chem. Ind.*, **1905**, 585.

The relative amounts of the various secondary constituents that are present, vary considerably in different kinds of distilled liquors, rum for example, being much higher in esters than is the case with whiskey, but the total amount is always much higher the simpler the process, that is, the less the amount of fractionation that the distillate undergoes. There is, of course no such thing in a commercial sense as absolutely pure alcohol, even the highest grades of cologne spirits containing measurable quantities of the secondary products, but the amount present is much less than in the case of such products as pot still whiskies. A better idea of this variation between various liquors and "silent spirit"[1] may be gained from Table LXXXIII, taken from Vasey's Analysis of Potable Spirits, and further examples are given later.

TABLE LXXXIII.—SECONDARY CONSTITUENTS IN DISTILLED SPIRITS

	Grams per 100 liters*					
	Volatile acids	Esters	Aldehydes	Furfural	Higher alcohols	Total
Genuine gin	0.0	37.3	1.8	0.0	44.6	83.7
Genuine rum	28.0	399.0	8.4	2.8	90.6	528.8
Cognac brandy, 10 years old	74.5	109.3	16.6	1.6	124.2	326.2
Genuine Scotch whiskey, 8 years old.	48.0	89.7	14.2	4.0	200.0	355.9
Patent spirit for whiskey blending.	8.4	23.8	4.9	0.35	trace	37.4
Sold as whiskey but probably patent spirit.	16.8	8.2	10.0	0.0	0.0	35.0
Brandy mixed with plain spirit	79.4	32.3	7.35	0.61	49.0	68.7

* These results are in grams per 100 liters of absolute alcohol, and should be divided by two to obtain the corresponding grams per 100 liters of 100° American proof. (See page 425.)

Forms of Adulteration.—From the viewpoint of methods of manufacture, as well as of differences in composition, whiskies may be broadly divided into four classes:[2]

[1] *Silent spirit, neutral spirit, velvet spirit, cologne spirit*, are all terms used to designate a distilled spirit from which practically all the constituents except ethyl alcohol and water have been separated.

[2] Shepard: *Tenth Ann. Proc. Assoc. Food & Dairy Dept's*, **1906**, 236.

1. Whiskey made wholly in a distillery, under government supervision, and usually by processes which produce little or no rectification. If sold as "bottled in bond," this product must be matured for at least four years in bonded warehouses and is sold at 100 per cent. proof and under a government stamp certifying that these conditions have been met. This whiskey is often referred to as "straight" whiskey, and owing to the length of time that it is held to mature is usually an expensive variety.

2. "Blended" whiskey, made by mixing two or more "straight" whiskies in such a manner that the flavor, body or other qualities may be varied to suit the requirements of the trade. These are also 100 per cent. proof and high in price.

3. The third class and by far the largest, is made by mixing a "straight" whiskey with silent spirit and water, usually adding some caramel in order to restore the deficiency in color. The terms "blended whiskey" and "rectified whiskey" are sometimes applied to this product, which is naturally cheaper to manufacture than the whiskies of the preceding classes.

4. Still another class of whiskey is wholly artificial, being made by adding water and coloring matter, together with various oils and essences to silent spirit. This product is of course not made under government supervision and it brings the lowest price in the market.

It should be recognized that the above classification is based upon the assumption that the straight or pot still whiskey is a higher grade or more "natural" product than that obtained from the fractionating still. For this point of view there is a strong argument in the fact that straight whiskey is the agent which the blender adds to the comparatively flavorless neutral or silent spirit in order to give it the characteristic flavor of whiskey.

On the other hand, as stated on page 469, it has been decided officially in both this country and Great Britain that the product of the fractionating still is as much entitled to the name whiskey, without qualification, as that obtained by the simpler and older process.

Under the terms of Food Inspection Decision 113, all unmixed distilled spirits from grain, colored and flavored with

harmless flavor and color, in the customary ways, either by the charred barrel process, or by the addition of caramel and harmless flavor, if of potable strength and not less than 80° proof, are entitled to the name whiskey without qualification. If the proof be less than 80°, *i.e.*, if more water be added, the actual proof must be stated on the label and this requirement applies as well to blends and compounds of whiskey.

Whiskies of the same or different kinds, *i.e.*, straight whiskey, rectified whiskey, re-distilled whiskey and neutral spirits whiskey are like substances, and mixtures of such whiskies, with or without harmless color and flavor used for purposes of coloring and flavoring only, are blends under the law and must be so labeled. It is required also that unmixed potable alcoholic distillates from sources other than grain, if sold as whiskey, shall be labeled "Imitation Whiskey." Further, that a whiskey of a particular flavor, as rye whiskey, if made by adding artificial flavor to the alcoholic distillate from another source, as corn, must be so labeled as to show that it is an imitation.

Under this conception of what constitutes a genuine whiskey, the chief form of adulteration would consist in a disagreement of the label with the actual contents of the package, that is, would be a mis-branding. Typical cases might be: (*a*) the refilling of a bonded or "double stamped package" with either an inferior grade of whiskey or with alcohol diluted to the proper proof and artificially colored and flavored; (*b*) the palming off of one variety of whiskey as a more expensive or more desirable kinds, such as labeling whiskey made of rectified or neutral spirits, colored and flavored, in such a way as to indicate that it was a straight rye or Bourbon whiskey; (*c*) incorrect or misleading statements as to the age of the whiskey or the materials from which it was made. It should be carefully borne in mind in attempting to show adulteration by chemical tests that whiskey is of itself a decidedly variable product, even more so under the terms of its legal definition; that in most cases it will be only the comparatively gross forms of adulteration that can be shown chemically, and that as regards the judging of quality or comparison of flavor more can be gained by a properly trained sense of taste than by any chemical analysis.

Methods of Analysis

Statement of Results.—Unless otherwise directed the results obtained should in each case be expressed as grams in 100 liters of *proof spirit*. For example, if 100 liters of whiskey of 88° proof contain 15.4 grams of aldehydes this would correspond to 17.5 grams per 100 liters of proof spirit.

Specific Gravity.—This is not often needed, but if desired may be determined by the pyknometer as described on page 3, taking care to avoid loss of alcohol by undue exposure or heating.

Alcohol.—Determine by distilling 25 cc. as directed on page 416. Report the result as per cent. by volume or degrees proof.

Note.—Since whiskey ordinarily contains only a slight proportion of extract, the determination of alcohol can be made more quickly and often with sufficient accuracy by means of a suitable hydrometer. The form prescribed by the Bureau of Internal Revenue, and accompanied by a complete set of temperature correction tables, is especially convenient. The presence of an appreciable amount of added sweetening would render the results less accurate.

Methyl Alcohol.—See page 426.

Extract.—Evaporate 50 cc. of whiskey to dryness on the water-bath, best in a platinum dish, dry for an hour at 100°C. and weigh.

Notes.—The official method of the Association of Official Agricultural Chemists specifies a sample of 100 cc. and a period of 2½ hours drying, but practically the same results are given by the shorter method.

Care should be taken not to expose the sample at first to the full heat of the bath, since on account of the high alcohol content vigorous boiling and consequent loss of sample may result.

Acids.—Titrate 50 cc. of the sample, diluted to 100 cc. with distilled water, with tenth-normal alkali, using phenolphthalein as indicator. Calculate the result in terms of acetic acid.

Esters.—Add 25 cc. of water to 200 cc. of the sample and distil slowly 200 cc., using a mercury valve to prevent loss of alcohol. Use 50 cc. of the distillate, exactly neutralize the free acid with tenth-normal alkali and phenolphthalein, add 25 to 50 cc. of tenth-normal alkali in excess and either boil for 1 hour with

a reflux condenser, cool and titrate with tenth-normal acid, or allow the solution to stand over night in a stoppered flask with the excess of alkali, heat with a tube condenser for half an hour at a temperature below the boiling point, cool and titrate. Carry out a blank determination at the same time, following exactly the same procedure. Calculate the grams of ethyl acetate corresponding to the number of cc. of tenth-normal alkali used to saponify the esters.

Notes.—Saponification is complete by standing over night in the cold with nearly all samples, but occasionally a low result is obtained, hence the additional precaution of heating for half an hour before titrating.

In very exact analyses it might be preferable to remove the aldehydes by treatment with *m*-phenylenediamine, as described below, but the error introduced by their presence is so slight that it may be neglected in practical work.

The mercury valve referred to consists of a small glass U tube with the bend sealed by a few drops of mercury. This is attached to the receiving flask by a two-holed rubber stopper, through the other opening of which passes the condenser tip. Thus a certain contraction and expansion of the contained air is permitted, while all loss is prevented. If the distillation is conducted slowly and the condenser tip extends some distance into the neck of the receiving flask, there is practically no loss even without such a valve.

The distillation must be conducted slowly, taking care especially not to overheat the sides of the distilling flask, to avoid decomposition and consequent formation of furfural.

Aldehydes.—*Preparation of Reagents.*—(*a*) *Aldehyde—free Alcohol.*—Use the best commercial alcohol obtainable and treat it with silver oxide as mentioned on page 158. After distilling from the silver oxide, add 3–5 grams of *m*-phenylenediamine hydrochloride per liter of alcohol, together with some glass beads, and boil it gently under a reflux condenser for several hours. Distil slowly, allowing the reagent to remain in the alcohol. Reject the first 50–100 cc. of the distillate. The remainder should give no color when tested with the fuchsin-sulphite reagent as described below, and may be preserved for some time in a cool dark place. The excess of *m*-phenylenediamine reagent may

be filtered off, washed with a little strong alcohol and dried on a porous tile, when it can be used again with the addition of a little fresh material.

(*b*) *Fuchsin-Sulphite Reagent.*—Dissolve 0.500 gram of pure powdered fuchsin in 500 cc. of warm water, cool, balance on a suitable scale and pass in sulphur dioxide until the weight has increased by 5 grams. Make up to a liter and allow to stand a few hours or until colorless.

Prepare only as much as needed, since the solution retains its strength for only a few days. Keep it in the ice-box.

(*c*) *Standard Aldehyde Solution.*[1]—Grind aldehyde ammonia in a mortar with ether and decant the ether. Repeat this operation several times, then dry the purified salt in a current of air and finally in a vacuum over sulphuric acid. Dissolve 1.386 grams of this purified aldehyde ammonia in 50 cc. of 95 per cent. alcohol, add 22.7 cc. of normal alcoholic sulphuric acid, then make up to 100 cc. and add 0.8 cc. of alcohol to compensate for the volume of the ammonium sulphate precipitate. Allow this to stand over night and filter. This stock solution contains 1 gram of acetaldehyde in 100 cc. and will retain its strength if kept cold and dark.

From this stock solution prepare when wanted a standard solution by diluting 2 cc. to 100 cc. with 50 per cent. alcohol. This dilute solution should be used the day it is made.

Determination.—Measure 10 cc. of the distillate obtained in the determination of esters into a 100 c..c Nessler tube, add 25 cc. of the aldehyde-free alcohol, 25 cc. of water, and immerse the tube in a bath of water kept at 15°C. At the same time prepare suitable standards from the standard aldehyde solution, say 0.5, 1.0, 2.0 and 4.0 cc., add the aldehyde-free alcohol and water as above and immerse all the tubes in the bath at 15°. Let stand 10 minutes in order to reach the temperature of the bath, then add rapidly to all the tubes 25 c.c of the fuchsin-sulphite reagent (which should also be at 15°C). Allow the tubes to stand in the bath for 15 minutes, then compare the colors. This may be done directly in the tubes or if a more accurate colorimeter, such as the Duboscq (page 20), be em-

[1] Vasey: The Analysis of Potable Spirits, p. 31.

ployed the solutions may be kept in the bath and transferred quickly to the colorimeter for reading.

Notes.—The treatment of the alcohol with *m*-phenylenediamine is to remove the last traces of aldehyde which are not taken out by the silver oxide, a non-volatile product being formed by condensation of the aldehyde with the two amino groups.

In the preparation of the fuchsin-sulphite reagent the amount of sulphur dioxide added is important, too much making the reagent less sensitive, and too little causing difficulty in getting a satisfactory "blank" with the alcohol itself. If difficulty should be experienced in preparing an alcohol which is absolutely aldehyde-free, it is possible to increase slightly the sulphur dioxide, say up to 6 grams per liter, without seriously interfering with the delicacy of the reaction.

The treatment of the aldehyde ammonia with ether is to remove the polymerized aldehyde resin present through partial decomposition. The purified product should be perfectly white and readily soluble in alcohol. This method of preparing a standard aldehyde solution is preferable to weighing out the aldehyde directly since the pure substance polymerizes so readily, thus changing in strength.

It is important in this determination that the temperature should be kept at 15° and that all the samples should be at the same temperature, since the color deepens greatly with a slight rise in temperature. It should be noted also that the color developed in the reaction is not directly proportional to the amount of aldehyde present, hence the reading of the sample should not be far different from that of the standard with which it is compared. If in any particular case this should be impracticable, the reading may be corrected by a table worked out by Tolman.[1]

Furfural, although an aldehyde, is not included in the determination on account of the very faint color it gives with the fuchsin-sulphite reagent as compared with acetic aldehyde.

Furfural.—*Standard Furfural Solution.*—Weigh 1 gram of re-distilled furfural and dissolve it in 100 cc. of 95 per cent. alcohol. This strong solution will keep (best cool and in the dark). Prepare the dilute solution for making the standards

[1] *J. Am. Chem. Soc.*, **1906**, 1627.

by diluting 1 cc. of this strong solution to 100 cc. with 50 per cent. (by volume) alcohol. Each cc. of this solution contains 0.0001 gram of furfural.

Determination.—Measure 10 or 20 cc. of the distillate obtained in the determination of esters into a 100 cc. Nessler tube, add 25 cc. of alcohol free from furfural,[1] 25 cc. of water and immerse the tube in a bath of water kept at 15°C. At the same time prepare suitable standards from the standard furfural solution, say 0.5, 1.0, 2.0 and 4.0 cc., add the furfural-free alcohol and water as above and immerse all the tubes in the bath at 15°. Let stand for 10 minutes, in order to reach the temperature of the bath, add to the tubes 2 cc. of colorless (redistilled) anilin, and then 0.5 cc. of hydrochloric acid (sp. gr. 1.125). Allow the tubes to remain in the bath for 15 minutes and then compare the colors as in the case of the aldehyde determination (page 479).

Notes.—The same reaction has been used as a qualitative test for furfural on pages 235 and 294, the only difference being that acetic acid was used instead of hydrochloric and no precautions were taken as regards the temperature. Acetic acid can be used just as well but in some instances has been found to contain traces of furfural or some impurity that reacts similarly. For quantitative tests the control of the temperature is highly important since it greatly affects the depth of color. At the low temperature chosen the reaction is perhaps less delicate but is more readily controlled.

Authorities differ as to whether the depth of color is directly proportional to the amount of furfural present, hence the strength of the sample should not be far different from that of the standard with which it is compared.

In order to avoid the possibility of forming furfural by decomposition during the distillation, Schidrowitz[2] has proposed to determine the furfural directly in the sample without distilling, removing the bulk of the color with lead acetate. This, however, works satisfactorily only with light colored samples, so that the test is best made on the carefully prepared distillate.

[1] This may be prepared by treatment of commercial alcohol with silver oxide, as mentioned on page 158.

[2] *J. Soc. Chem. Ind.*, **1902**, 815.

Higher Alcohols (Fusel Oil).—*Allen-Marquardt Method—Principle.*—Extraction of the higher alcohols by carbon tetrachloride, in which they are more readily soluble than is ethyl alcohol, washing of the carbon tetrachloride free from traces of ethyl alcohol, oxidation of the extracted alcohols of the fusel oil to the corresponding acids by potassium bichromate, distillation and titration of the acids formed.

Procedure.—Add to 100 cc. of whiskey 20 cc. of approximately half-normal sodium hydroxide and saponify the mixture by boiling for 1 hour under a reflux condenser. Connect the flask with a distilling apparatus, distil 90 cc., add 25 cc. of water, and continue the distillation until an additional 25 cc. is collected.

Approximately saturate the distillate with finely ground sodium chloride and add a saturated solution of sodium chloride until the specific gravity is 1.10, as determined by a small hydrometer or the Westphal balance.

Extract this salt solution four times with carbon tetrachloride, purified as described in the Notes below, using 40, 30, 20, and 10 cc. respectively, and wash the carbon tetrachloride three times with 50 cc. portions of a saturated solution of sodium chloride and twice with a saturated solution of sodium sulphate. Note the precautions given for quantitative extractions on page 94. Then transfer the carbon tetrachloride to a flask containing 5 cc. of concentrated sulphuric acid, 45 cc. of water, and 5 grams of potassium bichromate and boil for 8 hours under a tall reflux condenser.

Add 30 cc. of water and distil until only about 20 cc. remain; add 80 cc. of water and distil until a crust forms on the liquid and approximately only 5 cc. are left. Neutralize the distillate to methyl orange, which should not require more than 0.2 cc. of tenth-normal alkali, and titrate with tenth-normal sodium hydroxide, using phenolphthalein as indicator. Calculate the final titration to amyl alcohol.

Rubber stoppers can be used in the saponification and first distillation, but corks covered with tinfoil must be used in the oxidation and second distillation. Corks and tinfoil must be renewed frequently. A blank determination, which should not require more than 0.3 cc. of standard alkali, should be made from the beginning, using 100 cc. of 50 per cent. alcohol.

Notes.—It is essential that the carbon tetrachloride used be of the highest purity and it will generally be found necessary to purify the reagent. This may be done by boiling with potassium bichromate and sulphuric acid and distilling over barium carbonate, or more conveniently as follows:[1]

Mix the impure carbon tetrachloride with one-tenth its volume of strong sulphuric acid. Shake the mixture thoroughly at frequent intervals and allow it to stand over night. Then run water through the mixture continuously, by means of a glass tube inserted to the bottom of the bottle and connected with the water tap, until thoroughly washed free from acid and impurities. Draw off the upper layer of water by means of a siphon, the last portions being removed as far as possible with a pipette. Add an excess of soda solution and distil the carbon tetrachloride from it.

The endeavor should be made during the entire process to maintain uniform conditions so far as possible. The shaking should be with a long swinging motion, rather than quick and violent, and continued for 2 minutes each time. The solutions of sodium chloride and sodium sulphate should be perfectly saturated, best kept standing over some of the finely powdered salt. The temperature should be kept uniform and as low as convenient, since it has been found that a low temperature tends toward more efficient extraction. The boiling of the carbon tetrachloride with the oxidizing solution should be slow and regular, and a high condenser should be used to ensure the complete condensation of all the products.

The saponification with sodium hydroxide before distilling is for the purpose of holding back the esters and furfural, which would otherwise pass into the distillate. The washing of the carbon tetrachloride with saturated sodium chloride is to remove the small quantity of ethyl alcohol which is extracted along with the amyl alcohol. This must in turn be removed by thorough washing with sodium sulphate, since any chloride which remains will cause trouble through being oxidized to chlorine by the bichromate mixture and hence interfering with the titration by bleaching the indicator. A few drops of dilute sodium thiosulphate added just before the titration will be found an advantage. More than two washings with the sodium sulphate cannot be

[1] Breckler: *Bur. of Chem., Bull.* **122,** p. 209.

made without danger of removing some of the higher alcohols. Rubber stoppers should not be used during the oxidation and final distillation on account of the possibility of action by the oxidizing mixture; corks, on the other hand, are liable to absorb amyl alcohol and valeric acid, but can be used if carefully covered with tinfoil.

The titration with methyl orange as an indicator was originally supposed to take care of the mineral acids, chiefly hydrochloric, and consequently not to be included in the calculation of higher alcohols. It has been shown however, that it is probably due to some of the volatile fatty acids which are not absolutely neutral to methyl orange.[1] A preferable plan, unless the methyl orange acidity is excessive, would seem to be to calculate the total acidity to amyl alcohol, but such a change has not as yet met with general adoption.

The Allen-Marquardt method gives results which are undoubtedly somewhat below the truth. Fusel oil is a decidedly complex mixture and there is a certain error in calculating to amyl alcohol the propyl and butyl alcohols which are also present. Further, although the amyl alcohols are extracted practically quantitatively by carbon tetrachloride, the degree of extraction is distinctly less with the alcohols of lower molecular weight. It is true also that one of the alcohols present, iso-propyl alcohol, does not oxidize to volatile acid under the conditions of the determination, but to acetone. The method unquestionably gives, however, a reliable result in terms of an empirical standard, which is all that can be expected from the variable material being analyzed.

Of the other methods which have been proposed for this important determination, only two are used to any extent, the Roese-Herzfeld method being official in Germany and the colorimetric method of Girard and Cuniasse being quite largely used in France. In the first of these the fusel oil is determined by extracting under definite conditions with chloroform and noting the increase in volume of the latter. The conditions must be maintained and the reagents prepared with absolute exactness, so that the method is suited only for those of great skill and ex-

[1] Schidrowitz and Kaye: *Analyst*, **1906**, 183; Mann: *J. Soc. Chem. Ind.*, **1906**, 1125.

perience. The method, moreover, is not well suited for the examination of whiskey, since it has been shown that some of the constituents of whiskey may cause a contraction of the chloroform and consequently negative results.[1]

The French method is based on the color given with strong sulphuric acid by certain of the higher alcohols, isobutyl alcohol being chosen as the standard. The chief objection to this method is that the amyl alcohols, which are the most important ones in fusel oil, give very little color with sulphuric acid, and it has been stated that even the color given by isobutyl alcohol is due not to the alcohol itself but to impurities present.[2]

On the whole it may be safely said, especially when whiskey alone is being considered, that the only reliable method for determining the higher alcohols is the Allen-Marquardt, and this only when great care is exercised by the analyst.

For a further discussion of this important determination and a critical study of the methods the student is referred to the following papers:

Schidrowitz and Kaye: *Analyst*, **1905,** 190; **1906,** 181.

Mann: *J. Soc. Chem. Ind.*, **1906,** 1125.

Tolman and Hillyer: *Bur. of Chem., Bull.* **122,** p. 206.

Dudley: *J. Am. Chem. Soc.*, **1908,** 127.

Color.—While the degree of color present in whiskey has no special analytical significance, the character of the color is of great importance in judging the nature of the sample, since the color of straight whiskey is derived entirely from the wooden package in which it is stored, while that of compounds or imitations usually consists wholly or in part of caramel. The difference is best shown through certain considerations of solubility.

(*a*) *Color Insoluble in Water.*—Evaporate 50 cc. of the whiskey just to dryness on the water-bath, take up the residue in 10 cc. of hot distilled water and filter. Wash the paper thoroughly with small portions of water, make the filtrate up to 25 cc. with water and add enough alcohol to make 50 cc. (the original volume). Compare the color of this solution in a colorimeter with the untreated whiskey and calculate the per cent. of color remaining,

[1] Schidrowitz: *J. Soc. Chem. Ind.*, **1902,** 815.

[2] Veley: *J. Soc. Chem. Ind.*, **1906,** 398.

which subtracted from 100 is the percentage of color insoluble in water.

Note.—Caramel, the artificial color most commonly used in whiskey, is of course readily soluble in water, while *flavescein*, the coloring matter taken up by straight whiskey from the oak wood in which it is stored, is much less soluble. A genuine whiskey will seldom show less than 70 per cent. of color insoluble in water.

(*b*) *Color Insoluble in Amyl Alcohol.*—Evaporate 50 cc. of the whiskey just to dryness in a porcelain dish on the water-bath. Add 26.3 cc. of 95 per cent. alcohol to dissolve the residue and transfer to a 50 cc. flask. Wash out the dish and make up to the mark with water. Place 25 cc. of the 50 per cent. alcoholic solution in a separatory funnel, add 20 cc. of the Marsh reagent, and proceed as described on page 388 in detecting caramel in Vanilla Extract. Calculate the percentage of color not soluble in amyl alcohol.

Notes.—The test depends upon the relative solubility of coloring matters in ethyl alcohol, amyl alcohol and water. The addition of amyl alcohol, when in sufficient quantity, to a mixture of 50 parts of ethyl alcohol and 50 parts of water will cause a separation of the liquids into two layers, the lower layer being largely water and the upper one a mixture of ethyl alcohol, amyl alcohol and some water. As a result of this division, water-soluble coloring matter can be separated from alcohol-soluble coloring matter; that is, caramel can be separated from the natural coloring matter of whiskey.

In a straight American whiskey about 90 per cent. of the coloring matter is soluble in the amyl alcohol-ethyl alcohol layer, as compared with approximately 15 per cent. in the case of imitation whiskies. The method gives a sharper differentiation between caramel and the coloring matter taken from wood than the water-insoluble method even, and renders it possible to estimate approximately the amount of caramel that has been added to a mixture.

In the table given below[1] is seen the amount of caramel corresponding to the percentage of color insoluble in amyl alcohol determined by the method just described. This information is of

[1] Tolman: *Bur. of Chem., Bull.* **132,** p. 92.

value in that it shows the natural color of the whiskey before the caramel was added.

TABLE LXXXIV.—PER CENT. OF CARAMEL IN TOTAL COLOR OF WHISKEY CORRESPONDING TO COLOR INSOLUBLE IN AMYL ALCOHOL

Insoluble in amyl alcohol, per cent.	Due to caramel, per cent.	Insoluble in amyl alcohol, per cent.	Due to caramel, per cent.	Insoluble in amyl alcohol, per cent.	Due to caramel, per cent.
5.4	0.0	28	34.5	52	66.5
6.0	1.0	30	37.0	54	69.5
8.0	4.5	32	39.5	56	73.0
10.0	6.5	34	42.0	58	76.0
12.0	10.5	36	46.5	60	79.5
14.0	13.5	38	47.0	62	82.5
16.0	16.5	40	49.5	64	85.5
18.0	19.5	42	52.5	66	88.5
20.0	22.5	44	55.5	68	92.0
22.0	25.5	46	58.0	70	95.0
24.0	28.5	48	61.0	72	98.0
26.0	31.5	50	63.5	74	100.0

The test may be carried out somewhat more simply by shaking the original whiskey with amyl alcohol and estimating the per cent. of color soluble in the latter. In straight whiskey the per cent. of color soluble in amyl alcohol is usually over 70 per cent.

Qualitative Tests for Color.—These afford considerable information if several comparative tests are made on straight and imitation whiskies to gain the experience necessary to interpret the results.

(*a*) *Chloroform Test.*—Shake 5 cc. of the whiskey with an equal volume of chloroform. The greater part of the natural color will be dissolved by the chloroform, artificial colors, such as burnt sugar or coal-tar dyes, appearing in the upper layer.

(b) *Amyl Alcohol Test.*—Shake 5 cc. of the whiskey with 2 cc. of water and 3 cc. of amyl alcohol. The amyl alcohol dissolves most of the natural color, artificial colors being found in the aqueous or lower layer.

(c) *Paraldehyde Test.*—This may be carried out as described on page 56, omitting the preliminary precipitation with zinc hydroxide. In samples colored with caramel paraldehyde usu-

ally gives a marked turbidity within 10 minutes, observed best by holding the test-tube in front of and slightly below the source of light.

Interpretation of Results

It will be evident from what has been said already that the chemical analysis is best restricted to determining certain comparatively gross forms of adulteration.

The addition or substitution of neutral spirits, suitably colored and flavored, for straight whiskey, is a typical instance of this kind. It is a form of adulteration frequently practised, and will serve as a suitable illustration of the interpretation of the chemical analysis.

Authentic Analyses of Whiskey.—The most extended as well as instructive series of analyses of authentic samples of whiskey are undoubtedly those made under the direction of Crampton and Tolman[1] in a study of the changes that occur when whiskey is aged in wood. Thirty-one barrels of whiskey were kept in bonded warehouses for the aging period of eight years, a sample being removed each year and kept in glass until all were analyzed at the end of the bonded period. The maximum, minimum and average results obtained for various periods are shown in Table LXXXV. To eliminate some abnormal results, the next to the lowest figures are also included in most instances. The color is reported on the "brewers' scale" of the Lovibond tintometer on all the samples, hence gives comparative data for depth of color. The other determinations were made according to the methods described on pages 477 to 487. No caramel was found in any sample.

The results show a gradual increase in the constituents determined during the eight years. This is due to chemical changes and also, especially after the fourth year, to concentration, the water of the spirit, and to a lesser degree the alcohol, evaporating through the wood to a much greater extent than do the other constituents. The charring of the barrel has a pronounced effect on the body, color and flavor of the whiskey, samples which had been kept for eight years in uncharred barrels having

[1] *J. Am. Chem. Soc.*, **1908**, 98.

only as much of these characteristics as were produced by aging for two years in the charred package.

TABLE LXXXV.—COMPOSITION OF AMERICAN WHISKEY AT VARYING AGES

		Proof	Color	Grams per 100 liters of proof spirit					
				Extract	Acids	Esters	Aldehydes	Furfural	Fusel oil
Rye whiskey									
New	Average	**101.2**	**0.0**	**13.3**	**4.4**	**16.3**	**5.4**	**1.0**	**90.4**
	Maximum	102.0	0.0	30.0	72.0	21.8	15.0	1.9	161.8
	Minimum	100.0	0.0	5.0	12.0	4.3	0.7	trace	61.8 43.7
One year old	Average	**102.5**	**8.8**	**119.7**	**46.6**	**37.0**	**7.0**	**1.8**	**111.5**
	Maximum	104.0	13.8	171.0	60.5	64.8	15.5	3.3	194.0
	Minimum	101.0	7.2 6.6	93.0 92.0	31.1 5.8	6.8 6.8	2.8	0.4	80.4 66.4
Two years old	Average	**104.9**	**11.6**	**144.7**	**51.9**	**54.0**	**10.5**	**2.2**	**112.4**
	Maximum	109.0	16.7	199.0	75.6	75.1	18.7	5.7	214.0
	Minimum	100.0	8.8 8.6	121.0 94.0	44.3 11.0	41.5 31.2	5.4	0.7	83.4 82.2
Four years old	Average	**111.2**	**14.0**	**185.0**	**65.9**	**69.3**	**13.9**	**2.8**	**125.1**
	Maximum	118.0	18.9	238.0	83.8	89.1	22.1	6.7	203.5
	Minimum	105.0	11.6 11.3	156.0 153.0	58.6 17.3	57.7 36.3	6.4	0.7	83.8 67.8
Six years old	Average	**118.0**	**17.0**	**223.1**	**72.4**	**80.4**	**14.6**	**3.3**	**145.5**
	Maximum	132.0	21.2	284.0	95.8	109.0	22.3	8.3	245.3
	Minimum	110.0	13.7 12.4	193.0 176.0	67.1 24.3	64.0 39.1	7.3	0.7	99.2 80.0
Eight years old	Average	**123.8**	**18.6**	**256.0**	**82.9**	**89.1**	**16.0**	**3.4**	**154.2**
	Maximum	132.0	24.2	229.0	112.0	126.6	26.5	9.2	280.3
	Minimum	112.0	13.8 13.7	214.0 200.0	73.7 31.7	68.4 40.9	7.9	0.8	109.0 107.1
Bourbon Whiskey									
New	Average	**101.0**	**0.0**	**26.5**	**10.0**	**18.4**	**3.2**	**0.7**	**100.9**
	Maximum	104.0	0.0	161.0	29.1	53.2	7.9	2.0	171.3
	Minimum	100.0	0.0	4.0	12.0	13.0	1.0	trace	71.3 42.0
One year old	Average	**101.8**	**7.1**	**99.6**	**41.1**	**28.6**	**5.8**	**1.6**	**110.1**
	Maximum	103.0	10.9	193.0	55.3	55.9	8.6	7.9	173.4
	Minimum	100.0	5.4 4.6	61.0 54.0	24.7 10.4	17.2 10.4	2.7	trace	58.0 42.8
Two years old	Average	**102.2**	**8.6**	**126.8**	**45.6**	**40.0**	**8.4**	**1.6**	**110.1**
	Maximum	104.0	11.8	214.0	61.7	59.8	12.0	9.1	197.1
	Minimum	100.0	6.9 5.7	81.0 78.0	25.5 23.5	24.4 11.2	5.9	0.4	86.2 42.8
Four years old	Average	**104.3**	**10.8**	**151.9**	**58.4**	**53.5**	**11.0**	**1.9**	**123.9**
	Maximum	108.0	14.8	249.0	73.0	80.6	22.0	9.6	237.1
	Minimum	100.0	8.6 7.4	101.0 92.0	40.0 40.0	28.2 13.8	6.9	0.8	95.0 43.5
Six years old	Average	**107.9**	**13.1**	**185.1**	**67.1**	**64.0**	**11.9**	**1.8**	**135.3**
	Maximum	116.0	17.5	287.0	81.0	83.9	23.3	9.5	240.0
	Minimum	102.0	12.0 9.8	132.0 127.0	53.6 45.0	36.4 17.9	7.7	0.9	98.1
Eight years old	Average	**111.1**	**14.2**	**210.3**	**76.4**	**65.6**	**12.9**	**2.1**	**143.5**
	Maximum	124.0	20.9	326.0	91.4	93.6	28.8	10.0	241.8
	Minimum	102.0	12.3 10.5	152.0 141.0	64.1 53.7	37.7 22.1	8.7	1.0	110.0 47.6

For judging the character of a sample in the light of these analyses certain general considerations should be pointed out. The maximum and minimum figures are given only to show the range of values that may be expected and are not to be used directly in judging a whiskey since they do not coincide, except in one instance pointed out later. The fact that a given sample falls between the maximum and minimum figures given for a particular age is in itself no definite criterion of its genuine character. The average figures are of more value, but it is chiefly upon certain relationships that reliance must be placed.

Rye and Bourbon whiskies show certain characteristic differences, a typical one being the rate of increase in proof. This is accounted for by the fact that the rye whiskey is usually aged in heated warehouses, where the changes and evaporation taking place are aided by the higher temperature. In every other way the rye whiskies show this increased activity, containing more solids, color, acids, esters, etc.

The solids and color form the one exception noted above. Since with straight whiskey these are both derived entirely from the package it is to be expected that there should be a very close relationship between them. As a matter of fact, they show such similarity in rate of increase that it is possible to calculate quite closely from one what the other should be, a relationship which is of the greatest value in detecting artificial color in whiskey and deciding whether the solids are normal.

The relation between the acids and esters is important in distinguishing matured samples from young or imitation spirits. In the aging process the acids are formed more rapidly at first than the esters, but later the esters form more rapidly, so that the two reach an equilibrium in about 4 years. In the new whiskey the acids are distinctly lower than the esters, but at the end of the first year they are nearly the same and even higher, a relationship which gradually draws nearer until equilibrium is reached at the fourth year. In a given sample, then, a serious discrepancy in this respect would indicate that the whiskey was either not genuine or not properly matured.

In general, it may be said that a high color, high solids and high concentration should be accompanied by high acids and esters, and conversely, low color and solids go with low acids

and esters. Distinct discrepancies in this respect would create a suspicion that the sample was not a genuine straight whiskey.

Examples of commercial "bottled in bond" rye and Bourbon American whiskies are given in Table LXXVI, and analyses of genuine Scotch and Irish whiskies, taken from various authorities, in Table LXXVII.

TABLE LXXVI.—ANALYSES OF "BOTTLED IN BOND" AMERICAN WHISKIES

Source	Age	Grams in 100 liters of proof spirit					
		Solids	Acids	Esters	Aldehydes	Furfural	Fusel oil
Rye.....	8 years	189.8	79.2	81.8	17.5	3.0	84.9
Rye.....	7 years	212.0	79.5	94.0	22.5	5.0	119.5
Rye.....	7 years	280.0	93.1	86.1	10.3	4.5	162.0
Rye.....	8 years	222.0	91.7	87.6	11.9	4.7	116.0
Rye.....	4 years	174.0	75.7	71.0	9.7	1.1	194.0
Bourbon....	8 years	181.5	59.1	60.7	17.5	3.2	102.6
Bourbon....	4 years	148.5	54.9	55.9	15.0	2.6	152.0
Bourbon....	5 years	129.3	53.7	49.3	9.5	0.8	141.7
Bourbon....	7 years	220.0	88.2	81.4	10.3	2.5	153.0
Bourbon....	4 years	154.0	70.4	65.9	10.8	1.9	152.0

TABLE LXXXVII.—ANALYSES OF SCOTCH AND IRISH WHISKIES

Origin	Age	Grams per 100 liters[1]				
		Acids	Esters	Aldehydes	Furfural	Fusel oil
Scotch.....	New	25.4	61.9	11.4	6.2	199.4
Scotch.....	4 years	61.1	111.0	35.2	2.8	160.8
Scotch.....	8 years	48.0	89.7	14.2	4.0	200.0
Irish.....	New	20.9	7.6	6.5	0.4	174.0
Irish.....	8 years	41.8	20.9	11.2	3.4	204.0

It should not be understood, from the fact that the analyses of "genuine" whiskey which are given are all analyses of pot still spirit, that nothing else is properly entitled to be called whiskey. This has been previously pointed out on page 476. The analyses have been purposely selected because the substitution of straight whiskey by neutral spirits is the particular form of adulteration chosen for study.

[1] See note to Table LXXXIII, page 474.

Analyses of "Neutral" or "Silent" spirits or so-called "Blending Goods," typical of the distillates from rectifying stills which are used to mix with or substitute for straight whiskey, are given below, the figures in each case being reduced to 100 per cent. proof for better comparison with the analyses of whiskey previously quoted. Other examples are given on page 474.

	Grams per 100 liters of proof spirit					
	Solids	Acids	Esters	Aldehydes	Furfural	Fusel oil
1	5.1	3.2	3.9	1.2	0.0	15.0
2	...	13.3	20.4	0.5	0.0	6.3
3	1.6	2.4	32.1	5.5	0.0	19.8
4	...	2.5	3.6	0.1	0.0	2.9
5	2.4	7.2	26.4	6.0	0.0	28.0

The comparative ease with which the substitution of neutral spirits or inferior whiskey for a high-grade straight article can be detected, provided the analysis or characteristics of the original product are known, is shown by the following illustration taken from analyses made by the Internal Revenue Bureau preparatory to instituting legal proceedings for such a violation of the statutes.[1]

The possibility of doing this rests, of course, on whether the increase in color, solids and "congenerics" which results on the usual storage is sufficiently uniform to be comparable in different packages of approximately the same age. Exhaustive series of analyses made by the Internal Revenue Bureau show conclusively that this is possible, the variations in composition of the whiskey from different barrels kept under the same conditions being no greater than those due to the analytical methods themselves. The individual packages affect only the depth of color (not its composition) and to a less degree the amount of aldehydes and furfural. Many analyses and a valuable discussion of the points brought out will be found in the original paper, the figures given below being typical of the differences found.

The samples suspected of not being genuine are No. 5 in the first group and No. 10 in the second. The figures show plainly

[1] Adams: *J. Ind. Eng. Chem.*, **1911**, 647.

TABLE LXXXVIII.—COMPARISON OF GENUINE AND ADULTERATED WHISKIES

No.	Approx. age	Proof	Calculated to proof							Water-insoluble color	Color soluble in amyl alcohol	Genuine color calculated	Qual. color tests (p. 487)	Sum of congenerics
			Solids	Color	Acids	Esters	Aldehydes	Furfural	Fusel oil					
1	6 yrs.	109	190	13.8	79.3	79.9	17.0	1.7	170	79	76	10.5	Genuine	347.9
2	6 yrs.	109	191	14.0	88.0	79.9	17.0	1.8	179	74	80	11.2	Genuine	365.7
3	6 yrs.	109	202	14.5	81.5	75.5	15.6	1.8	208	76	80	11.6	Genuine	382.4
4	6 yrs.	109	199	14.7	87.0	80.4	14.7	2.0	198	75	78	11.4	Genuine	382.1
5	**6 yrs.**	**109**	**136**	**12.8**	**25.3**	**29.7**	**4.2**	**0.6**	**67**	**9**	**26**	**3.3**	**Caramel heavy**	**126.8**
6	4 yrs.	104	153	10.6	76.1	67.7	11.2	1.1	142	77	87	9.2	Genuine	298.1
7	4 yrs.	105	167	13.1	84.6	79.1	11.0	1.4	163	77	76	10.0	Genuine	337.1
8	4 yrs.	105	167	13.3	81.1	69.1	12.2	1.5	137	74	76	10.1	Genuine	298.9
9	4 yrs.	105	151	12.4	76.6	70.5	11.0	1.2	132	74	80	9.9	Genuine	291.3
10	**4 yrs.**	**105**	**125**	**12.4**	**21.7**	**26.1**	**2.3**	**trace**	**51**	**00**	**17**	**2.1**	**Caramel heavy**	**101.1**

the close agreement in essentials among the genuine samples kept under the same conditions of storage, and also that the chief points of resemblance between the genuine and suspected samples are in the proof and amount of color. These are, of course, easily adjusted at will to suit any conditions desired. In all other respects there is a marked discrepancy, this being especially noticeable in the acids, esters and fusel oil, the latter being only about one-third as much in the suspected samples. The great differences in the character of the color, as shown by the proportion insoluble in water and the per cent. soluble in amyl alcohol should be noted. The qualitative tests indicated the color of the genuine samples to be natural, derived from the oak wood; that of the suspected whiskey was largely artificial. The sum of the congenerics was again only one-third as much in the suspected as in the genuine samples. The acids, esters and composition of the color formed the main reliance of the chemist in prosecuting the suit.

Finally, the following analyses, taken from the records of actual cases[1] bear out the contention of the chemist that the products in question, labeled as being "Pure Straight Whiskey" —"100 Proof Guaranteed Straight Whiskey"—"No Blend. No Compound. No Imitation" were not properly so marked.

[1] Food and Drugs Act, Notices of Judgment, 343 and 3604.

	Proof	Color insoluble in amyl alcohol, per cent.	Water-insoluble color, per cent.	Grams per 100 liters of proof spirit					
				Solids	Acids	Esters	Aldehydes	Furfural	Fusel oil
A.	75.5	72.0	0.0	647.4	23.0	15.8	2.0	0.13	37.0
B.	100.3	70.0	...	129.6	12.0	8.8	2.4	0.2	28.1

Adulteration and misbranding were alleged on the ground that the product was not a pure straight whiskey but an article consisting of neutral or re-distilled spirits, artificially colored in imitation of straight whiskey and in one instance reduced with water to a much lower proof than pure straight whiskey. A verdict was rendered in each case favorable to the Government.

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